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WASHINGTON, D.C. 20460

Office of Prevention, Pesticides
and
Toxic Substances

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MEMORANDUM

SUBJECT: **CARBARYL**: **UPDATED TOXICOLOGY CHAPTER FOR RED**

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Attached is the Updated Toxicology Chapter for Carbaryl for the RED. It replaces the chapter dated December 7, 1999.

CARBARYL

PC Code: 056801

Updated Toxicology Disciplinary Chapter for the Reregistration Eligibility Decision Document

Date completed: May 24, 2002

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1.0 HAZARD CHARACTERIZATION

The toxicology data base is of good quality and is essentially complete. A 90-day inhalation study with cholinesterase measurements is required. The database provides sufficient information for selecting toxicity endpoints for risk assessment and therefore, supports a reregistration eligibility decision for the currently registered uses.

Carbaryl is a carbamate insecticide. Its primary mode of toxic action is through cholinesterase inhibition (ChEI) after single or multiple exposures. In most of the toxicology studies in which ChE was measured, it was the endpoint used to set the Lowest Observed Adverse Effect Level (LOAEL).

The acute toxicity studies showed that carbaryl was relatively toxic with acute oral dosing (Tox. Category II); but the acute dermal and inhalation toxicities were low (Tox. Categories III and IV, respectively). Carbaryl was not a dermal or eye irritant and was not a dermal sensitizer.

The neurotoxicity data showed that carbaryl was not a delayed neurotoxicant in the hen. In the acute neurotoxicity study in the rat after a single dose of 10 mg/kg carbaryl, ChEI was observed in plasma, whole blood, red blood cells (RBC) and brain. At the next higher dose (50 mg/kg), clinical signs typical of carbamate toxicity were observed. In the subchronic neurotoxicity study after 90 days of administration, clinical signs of toxicity were seen at the same dose (10 mg/kg/day) as plasma, whole blood, RBC and brain ChEI. There was no evidence of structural neuropathology in these studies.

No subchronic studies in the rat or dog are available, except for the subchronic neurotoxicity study in rats and 4-week dermal toxicity studies in rats (one with technical chemical and two with formulations). One of the dermal toxicity studies was useful for risk assessment. In this study, the systemic NOAEL was 20 mg/kg/day based on decreased RBC ChE in males and females and brain ChE in males at 50 mg/kg/day. The chronic toxicity data showed that, in dogs, decreases in plasma, RBC and brain ChE were observed at 10 mg/kg/day; clinical signs of toxicity were also observed in both sexes at 31 mg/kg/day. Brain and plasma ChE were decreased in female dogs at 3.1 mg/kg/day. In the mouse, clinical signs of toxicity were not typical of ChEI, but there was ChEI (plasma, RBC and brain) at 146 mg/kg/day. In the chronic toxicity study in rats, carbaryl at the highest dose (350 mg/kg/day in males and 485 mg/kg/day in females) caused a variety of toxic effects in the liver, kidneys and urinary bladder. It also induced an increase in the incidence of thyroid follicular cell hypertrophy and degeneration of sciatic nerves and skeletal muscle. RBC ChE was decreased in males at 60 mg/kg/day and in females at 79 mg/kg/day. The lowest LOAEL in the chronic studies was in the chronic dog study, i.e., 3.1 mg/kg/day, which was the lowest dose in females. In a follow-up 5-week study in dogs to clarify the NOAEL for ChEI, plasma ChE was decreased in males at 3.83 mg/kg/day; no effects were observed at 1.43 mg/kg/day.

In a prenatal developmental toxicity study in the rat, maternal toxicity was observed at the same dose (10 mg/kg/day) as developmental toxicity; the NOAEL was 4 mg/kg/day. Developmental effects included decreased fetal body weight and increased incomplete ossification of multiple bones. In a prenatal developmental toxicity study in the rabbit, the maternal and developmental LOAELs were 50 mg/kg/day and 150 mg/kg/day, respectively. The respective NOAELs were 5 mg/kg/day and 50 mg/kg/day. The only evidence of developmental toxicity was a decrease in fetal body weight. These studies showed no evidence of a qualitative or quantitative increased susceptibility. In the reproduction study, there was evidence of a quantitative offspring susceptibility. The LOAEL for parental systemic toxicity was 1500

ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females) based on decreased body weight, weight gain, and feed consumption. The NOAEL was 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females). The LOAEL for offspring toxicity was 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females) based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival. The NOAEL was 75 ppm (4.67-5.79 mg/kg/day for males and 5.56-6.41 mg/kg/day for females). In the developmental neurotoxicity study, there was evidence of qualitative susceptibility. Clinical signs of toxicity and plasma and brain ChEI were seen in maternal animals at the same dose (10 mg/kg/day) as changes in brain morphometric measurements (decreases in cerebellar measurements in females on Day 11 post-partum) were observed in offspring; however, brain measurements were not conducted at the next lower dose.

The Health Effects Division's (HED) Cancer Assessment Review Committee (CARC)(11/7/01) classified carbaryl as Likely to be carcinogenic in humans based on an increased incidence of hemangiosarcomas in male mice at all doses tested (100, 1000 and 8000 ppm). The Q_1*, based on the CD-1 mouse dietary study with ¾ Interspecies Scaling Factor, is 8.75×10^{-4} $(mg/kg/day)^{-1}$ in human equivalents. In addition to the required carcinogenicity studies in mice and rats, the registrant submitted a special study in genetically modified mice. Carbaryl was administered to heterozygous p53-deficient (knockout) male mice in the diet at concentrations of up to 4000 ppm (716.6 mg/kg/day) for six months. There was no evidence of neoplastic or preneoplastic changes in the vascular tissues of any organ. A model validation study demonstrated that vascular tumors occur in heterozygous p53 deficient mice within six months of administration of a known genotoxic carcinogen (urethane).

A recent review of the data from the submitted studies and the published literature show that carbaryl is clastogenic *in vitro.* The wide variety of induced aberrations (both simple and complex) was consistent between the submitted micronucleus study and the open literature. However, there are inconsistencies relative to the requirement for S9 activation. Nevertheless, the two *in vivo* studies for micronuclei induction or chromosome aberrations were negative. Similarly, the 6-month p53 knockout transgenic mouse bioassay was negative. Carbaryl was also negative for DNA binding in the livers of mice treated with 8000 ppm for 2 weeks. Metabolism studies identified epoxide intermediates of carbaryl which were found to be conjugated to glucuronide, rapidly metabolized and excreted as any endogenous epoxide would be. Overall, these findings indicate that carbaryl produces epoxides and its DNA reactivity is manifested as chromosomal aberrations in cultured mammalian cells. Other *in vitro* studies indicate carbaryl's effects on karyokinesis and cytokinesis, as well as stress genes associated with oxidative damage. Based on these considerations, the CARC concluded that there is a concern for mutagenicity, which is somewhat lessened because of the lack of an effect in *in vivo* mutagenicity studies.

The metabolism data in the rat indicated that radiolabeled carbaryl was readily absorbed with oral dosing, distributed to various organs, metabolized and formed conjugated metabolites with compounds such glucuronic acid. A total of 20 components was found, and 2 major metabolites were identified, naphthyl sulfate and naphthyl glucuronide. Much of the radioactivity was eliminated within 24 hours after dosing (86% in urine and 11% in feces). Seven days post dosing, negligible amounts of the administered dose were found in tissues. Several special metabolism studies were conducted to explore a mechanism for the increase in tumor incidence in mice. The results appear to show that high doses of carbaryl treatment (1154 mg/kg) led to a "phenobarbital" type of induction of liver xenobiotic-metabolizing enzymes and

interaction of carbaryl with chromatin protein in mice.

A dermal absorption study indicated that 12.7% of a carbaryl formulation (43.9% a.i.) was absorbed systemically.

2.0 REQUIREMENTS

The requirements (CFR 158.340) for food use for CARBARYL are in Table 1. Inclusion of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Table 1. Carbaryl - Data Requirements

Test	Technical	
	Required	**Satisfied**
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (rodent)	yes	yes[a]
870.3150 Oral Subchronic (nonrodent)	yes	yes[b]
870.3200 21-Day Dermal	yes	yes[c]
870.3250 90-Day Dermal	no	no
870.3465 90-Day Inhalation	yes	no
870.3700a Developmental Toxicity (rodent)	yes	yes
870.3700b Developmental Toxicity (nonrodent)	yes	yes
870.3800 Reproduction	yes	yes
870.4100a Chronic Toxicity (rodent)	yes	yes[b]
870.4100b Chronic Toxicity (nonrodent)	yes	yes
870.4200a Oncogenicity (rat)	yes	yes[b]
870.4200b Oncogenicity (mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes
870.5100 Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300 Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5385 Mutagenicity—Structural Chromosomal Aberrations	yes	yes[d]
870.5550 Mutagenicity—Other Genotoxic Effects	yes	yes
870.6100a Acute Delayed Neurotox. (hen)	yes	yes
870.6100b 90-Day Neurotoxicity (hen)	no	no
870.6200a Acute Neurotox. Screening Battery (rat)	yes	yes
870.6200b 90 Day Neuro. Screening Battery (rat)	yes	yes
870.6300 Develop. Neuro	yes	yes
870.7485 General Metabolism	yes	yes
870.7600 Dermal Penetration	yes	yes
Special Studies for Ocular Effects		
Acute Oral (rat)	no	
Subchronic Oral (rat)	no	
Six-month Oral (dog)	no	

a Satisfied with chronic toxicity study

b Satisfied with combined chronic toxicity/carcinogenicity study

c Satisfied with 4-week non-guideline study which was satisfactory for risk assessment

d Micronucleus study required by the CARC was unacceptable because the doses were not high enough. However, two studies from the open literature tested carbaryl up to the LD_{50} or 1/3 of the LD_{50}, which was higher than the high dose in the submitted study and negative.

3.0 DATA GAP(S)

90-day inhalation study with cholinesterase measurements

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of data base for acute toxicity: The data base for acute toxicity is considered complete. No additional studies are required at this time. The chemical is moderately acutely toxic by the oral route (Toxicity Category II), relatively nontoxic by the dermal and inhalation routes (Toxicity Category III and IV, respectively), not a primary eye or skin irritant or a dermal sensitizer.

The acute toxicity data on CARBARYL Technical is summarized below in Table 2.

Table 2. Acute Toxicity Data on CARBARYL

Guideline No.	Study Type	MRIDs #	Results	Toxicity Category
81-1	Acute Oral - rat	00148500	LD_{50} for males = 302.6 mg/kg; for females = 311.5 mg/kg; combined = 301.0 mg/kg	II
81-2	Acute Dermal - rabbit	00148501	LD_{50} > 2000 mg/kg	III
81-3	Acute Inhalation - rat	00148502	LC_{50} > 3.4 mg/L	IV
81-4	Primary Eye Irritation	00148503	not a primary eye irritant	IV
81-5	Primary Skin Irritation	00148504	not a primary skin irritant	IV
81-6	Dermal Sensiti-zation	00148505	negative	

4.2 Subchronic Toxicity

Adequacy of data base for Subchronic Toxicity: There are no acceptable subchronic toxicity studies in rodents or nonrodents. However, there are acceptable chronic studies, including a chronic toxicity study in the dog and a combined chronic toxicity/carcinogenicity study in the rat. Therefore, the requirements for subchronic toxicity studies in dogs and rats can be waived. There is an acceptable subchronic neurotoxicity study in the rat (discussed under G. Neurotoxicity). Three 4-week non-guideline

dermal toxicity studies in the rat were conducted. One with technical carbaryl was classified as acceptable/non-guideline and was used for the risk assessment. The other two with formulations were classified as unacceptable/non-guideline. No additional dermal toxicity studies are required at this time.

MRID 45630601

In a non-guideline four-week dermal toxicity study (MRID 45630601), Carbaryl Technical (99.49% a.i., Lot 211048078) was applied to the shaved skin of 10 Crl: CD (SD)IGS BR rats/sex/dose at dose levels of 0, 20, 50 or 100 mg/kg bw/day, 6 hours/day for 5 days/week during a 4-week period. The parameters measured included the following: clinical observations, body weight, body weight gain, food consumption, RBC and brain cholinesterase and signs of dermal irritation.

There was no treatment-related effect on mortality, clinical observations, body weight or dermal irritation. The only statistically significant body weight gain changes were a decrease (27%) in the 100 mg/kg/day males during Days 5 to 12 and an increase (37%) in 50 mg/kg/day males during Days 19 to 26. However, there were non-significant decreases in the 100 mg/kg/day males at Days -3 to 5 (16%), 12 to 19 (17%) and -3 to 26 (12%) which are considered toxicologically significant.

The only statistically significant decreases in food consumption were in the 50 mg/kg/day females on Days 12 to 19 and 50 and in the 100 mg/kg/day females on Days 19 to 26. The effects are not considered treatment-related as there was no dose-response and the decreases were minimal (9% and 8% in the 50 and 100 mg/kg/day groups, respectively).

RBC cholinesterase was measured before dosing on Day -4 and on Days 1, 8, 15 and 22. The only statistically significant effects were in the 100 mg/kg/day males at Days 8 (11% decrease) and 22 (13%). Using the repeated measures statistical test, there were also significant decreases in the 50 and 100 mg/kg/day females (11% and 10%, respectively) on Day 22. These effects were determined to be not toxicologically significant because they were inconsistent.

Measurements were also performed within 1 hour after test material removal on Days 5, 12, 19, and 26. Statistically significant decreases were observed in the 50 mg/kg/day (12% decrease) and 100 mg/kg/day (15%) males on Day 5 and in the 100 mg/kg/day males on Days 12 (21%) and 19 (16%). Using the repeated measures statistical test, there was also a significant decrease (10%) in the 50 mg/kg/day males on Day 12. In females, statistically significant decreases were observed in the 50 and 100 mg/kg/day groups on Days 5 (13% and 12%, respectively) and Day 12 (20% and 13%, respectively).

Brain cholinesterase was statistically significantly decreased in the 50 mg/kg/day males (15%) and in the 100 mg/kg/day males (15%) and females (24%). There was also a non-significant decrease in the 50 mg/kg/day females (9%).

The systemic LOAEL is conservatively established at 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females and brain cholinesterase in males. The systemic NOAEL is 20 mg/kg/day.

The dermal LOAEL was not established. The dermal NOAEL was 100 mg/kg/day.

This 4-week dermal toxicity study in the rat is **acceptable (non-guideline)**. The study was intended to establish endpoints for short-term and intermediate-term occupational and residential postapplication dermal exposure. Although the study does not meet guideline requirements, it is useful for risk assessment for the following reasons: 1) in all oral studies in which cholinesterase was measured, it was the most sensitive endpoint; therefore, other guideline parameters would most likely not establish a lower LOAEL; 2) plasma cholinesterase was not measured; however, in all the oral studies in rats, all three compartments (plasma, RBC and brain) were affected at the same dose level. Therefore, it is likely that plasma cholinesterase would not have been inhibited at a lower level, especially given the minimal effects on RBC and brain cholinesterase.

MRID 45630602

In a non-guideline four-week dermal toxicity study (MRID 45630602), Sevin® XLR Plus (44.82% a.i., Lot 60618902) was applied to the shaved skin of 8 Crl: CD (SD)IGS BR rats/sex/dose at dose levels of 0, 20, 50 or 100 mcL/kg bw/day (0, 9.6, 24 or 48 mg/kg/day), 6 hours/day for 5 days/week during a 4-week period. The parameters measured included the following: clinical observations, body weight, body weight gain, food consumption, RBC cholinesterase and signs of dermal irritation.

There were no treatment-related effects on clinical observations or body weight or evidence of dermal irritation. Females treated at 100 mcL/kg/day gained 167%, 65%, 144% and 40% of control values for Days -3 to 5, 5 to 12, 12 to 19 and 19 to 26, respectively. Overall (Days -3 to 26) body weight gain was not affected. It is difficult to determine if there was a treatment-related effect immediately after dosing as the first body weight measurement was not done until Day 5 of dosing. Although not statistically significant, there does appear to be a treatment-related decrease on the body weight gain (Days 5 to 12) of females treated at 100 mcL/kg/day.

RBC cholinesterase was measured before dosing on Week -1 and on Days 1, 8, 15 and 22. There was no evidence of a treatment-related effect at these time periods. Measurements were also performed within 1 hour after test material removal on Days 5, 12, 19, and 26. In males, the only statistically significant difference from control values was on Day 26 in the animals dosed at 50 mcL/kg/day; the decrease was only 8%. Although not statistically significant, the RBC cholinesterase on Day 12 in males treated at 100 mcL/kg/day was decreased by 10%. In females treated at 100 mcL/kg/day, values were significantly decreased on Day 5 (12%), Day 12 (12%) and non-significantly decreased on Days 19 (5%) and Day 26 (7%). There were also significant decreases in the 50 mcL/kg/day females on Days 19 (9%) and 26 (14%) and in the 20 mg/kg/day group on Day 26 (10%). Although statistically significant, the RBC cholinesterase decreases are not judged to be toxicologically significant due to the small magnitude of the effect and the lack of a dose-response on Days 19 and 26.

The systemic LOAEL in females was 100 mcL/kg/day (48 mg/kg/day) based on decreased body weight gain. The systemic NOAEL was 50 mcL/kg/day (24 mg/kg/day). The systemic LOAEL in males was not established. The systemic NOAEL was 100 mcL/kg/day (48 mg/kg/day).

The dermal LOAEL was not established. The dermal NOAEL was 100 mcL/kg/day (48 mg/kg/day).

This 4-week dermal toxicity study in the rat is **unacceptable (non-guideline)**. The study was intended for use in the short-term and intermediate-term occupational and residential handler risk assessments for

the liquid formulations of carbaryl. It is considered unacceptable and **not upgradeable** because RBC cholinesterase results were inconsistent and plasma and brain cholinesterase were not measured. In another dermal toxicity study (MRID 45630601), brain cholinesterase inhibition was the most sensitive and reliable endpoint. Determination of cholinesterase inhibition in all three compartments would have helped define the effect level.

MRID 45630603

In a non-guideline four-week dermal toxicity study (MRID 45630603), Sevin® 80S (80.07% a.i., Lot C8I168025A) was applied to the shaved skin of 8 Crl: CD (SD)IGS BR rats/sex/dose at dose levels of 0, 20, 50 or 100 mg/kg bw/day, 6 hours/day for 5 days/week during a 4-week period. The parameters measured included the following: clinical observations, body weight, body weight gain, food consumption, RBC cholinesterase and signs of dermal irritation.

There was no treatment-related effect on mortality, clinical observations, body weight or dermal irritation. Body weight gain (relative to control values) in the 100 mg/kg/day males was highly variable between time periods. There were non-significant decreases of 15% and 20% on Days -3 to 5 and 19 to 26, respectively but increases of 9% and 53% were observed on Days 5 to 12 and 12 to 19, respectively. Since body weight was not measured at treatment initiation, it is difficult to determine if there was an effect during the first time period. However, food consumption was significantly decreased by 12% on Days -1 to 5 in this group, which correlates with an initial treatment-related effect. Therefore, the decrease in body weight gain in the 100 mg/kg/day males is considered treatment-related.

RBC cholinesterase was measured before dosing on Week -1 and on Days 1, 8, 15 and 22. Statistically significant decreases were observed in the 50 and 100 mg/kg/day females on Day 8 (10% and 12%, respectively). These effects are not considered toxicologically significant given the inconsistency of the findings. Measurements were also performed within 1 hour after test material removal on Days 5, 12, 19, and 26. In males, statistically significant decreases were observed in animals treated at 50 mg/kg/day on Days 12 (10%), 19 (13%) and 26 (8%). In males treated at 100 mg/kg/day, there were significant decreases on Days 12 (20%), 19 (19%) and 26 (19%). Although not statistically significant, there was also a 12% decrease on Day 5 in this group. In females, statistically significant decreases were observed in animals treated at 50 mg/kg/day on Days 12 (16%) and 19 (12%). Using the repeated measures ANOVA test, there was also a significant decrease on Day 5 (12%) in this group. In females at 100 mg/kg/day, there were significant decreases on Days 12 (18%) and 19 (15%) and Day 26 (15%).

The systemic LOAEL is 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females. The systemic NOAEL is 20 mg/kg/day.

The dermal LOAEL was not established. The dermal NOAEL was 100 mg/kg/day.

This 4-week dermal toxicity study in the rat is **unacceptable (non-guideline)**. The study was intended for use in the short-term and intermediate-term occupational and residential handler risk assessments for the solid formulations of carbaryl. It is considered unacceptable and not upgradeable because only RBC cholinesterase was measured. In the dermal toxicity study with the technical chemical (MRID 45630601), brain cholinesterase inhibition was the most sensitive and reliable endpoint. While this study does support the RBC cholinesterase effects in MRID 45630601, the lack of plasma and brain cholinesterase

measurements makes the study unacceptable for use in risk assessment.

4.3 Prenatal Developmental Toxicity

Adequacy of data base for Prenatal Developmental Toxicity: The data base for prenatal developmental toxicity is considered complete. No additional studies are required at this time. There are acceptable prenatal developmental toxicity studies in the rat and rabbit. There was no evidence of increased fetal susceptibility in these studies.

870.3700a Prenatal Developmental Toxicity Study - Rat

In a developmental toxicity study (MRID 44732901), Carbaryl (99% a.i.) in an aqueous methylcellulose suspension was administered by gavage at 0, 1, 4, and 30 mg/kg/day to pregnant Crl: CD (SD) BR rats (25/dose) during gestation days (GDs) 6 through 20. At GD 21, surviving dams were sacrificed and necropsied.

There were no treatment-related gross pathologic findings noted in any of the dams. There were no differences of toxicological concern in mortality, pregnancy rate, numbers of corpora lutea, implantations, viable fetuses, pre- and post-implantation losses, placental weights, and sex ratio.

At 30 mg/kg/day, at least one occurrence of post-dosing salivation occurred in 18/25 of the dams (vs 0/25 controls). This clinical sign appeared within 20 minutes of treatment, disappeared after approximately one hour, and was observed from GD 13 to 20. There were no deaths and no other treatment-related clinical signs. Body weights of the high-dose dams were 3-8% less than controls throughout the study (not statistically significant); their corrected (for gravid uterine weight) body weights and body weight gains were decreased ($p \leq 0.01$) by 7 and 38%, respectively. Body weight gains in this group were decreased immediately after initiation of dosing (GDs 6-9, $\downarrow$108%, $p \leq 0.01$) and throughout treatment (overall, $\downarrow$27%, $p \leq 0.01$). Food consumption (g/animal/day) was decreased throughout the treatment period ($\downarrow$10-17%, $p \leq 0.01$).

There were no differences of toxicological concern observed in the mid- and low-dose groups.

The maternal LOAEL is 30 mg/kg/day based on clinical signs of toxicity, decreased body weight gains and food consumption. The maternal NOAEL is 4 mg/kg/day.

In the high-dose fetuses, mean fetal body weights were reduced ($\downarrow$7-8%, $p \leq 0.01$). Additionally, the following were observed in the high-dose male and female fetuses: (I) an increase in incomplete ossification of the 5th sternebra, (ii) unossified 7th cervical centrum, (iii) incomplete ossification of 7th cervical centrum, and (iv) unossified 1st metatarsal. No effects on fetal viability were observed.

There were no treatment related effects in developmental parameters observed in the mid- and low-dose groups.

The developmental LOAEL is 30 mg/kg/day based on decreased fetal body weights and increased

incomplete ossification of multiple bones. The developmental NOAEL is 4 mg/kg/day.

The developmental toxicity study in the rat is classified as **acceptable (§83-3(a))** and **satisfies** the guideline requirement for a developmental toxicity study in the rat.

870.3700b Prenatal Developmental Toxicity Study - Rabbit

In a developmental toxicity study (MRID 44904202), carbaryl (99% a.i.) in an aqueous methylcellulose suspension was administered by gavage at doses of 0, 5, 50 or 150 mg/kg/day to pregnant New Zealand White rabbits (22/dose) during Gestation Days (GD) 6-29. On GD 25, blood was collected 1 hour post-dosing for plasma and red blood cell (RBC) cholinesterase (ChE) measurements. At GD 30, surviving dams were sacrificed and necropsied; fetuses were examined for evidence of developmental effects. Maternal toxicity at 150 mg/kg/day was observed as statistically significant decreased body weight gain as compared to the control value during GD 6-9 (208%), GD 6-29 (dosing period, 53%), GD 3-30 (33%) and gestation (GD 0- GD 30, 38%). Corrected body weight change was also decreased at this dose (-219.73 g vs -81.86 g in the control). Although not statistically significant, the body weight decreases at 50 mg/kg/day can be considered biologically significant for GD 6-9 (55%), GD 6-29 (25%), GD 3-30 (14%) and gestation (14%). There was no treatment-related effect on food consumption. Statistically significantly decreases in plasma (46-68%) and RBC (19-27%) ChE were seen at 50 and 150 mg/kg/day.

Maternal LOAEL = 50 mg/kg/day based on decreased body weight gain and decreased plasma and RBC ChE; Maternal NOAEL = 5 mg/kg/day

The only evidence of developmental toxicity was a statistically significant decrease in fetal body weights of 10% (when calculated for all fetuses or individually for males and females) at 150 mg/kg/day. There were no treatment-related developmental effects observed in the mid- and low-dose groups.

Developmental Toxicity LOAEL is 150 mg/kg/day based on decreased fetal weight.
Developmental Toxicity NOAEL is 50 mg/kg/day

The developmental toxicity study in the rabbit is classified as acceptable/guideline and does satisfy the guideline requirement for a developmental toxicity study in the rabbit.

4.4 Reproductive Toxicity

Adequacy of data base for Reproductive Toxicity: The data base for reproductive toxicity is considered complete. No additional studies are required at this time. In the reproduction study in rats, there was evidence of quantitative susceptibility of offsprings. The LOAEL for parental systemic toxicity was based on decreased body weight, weight gain, and feed consumption; the NOAEL was 27 mg/kg/day in males and 30 mg/kg/day in females. In the offspring the LOAEL was based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival; the NOAEL was 5 mg/kg/day in males and 6 mg/kg/day in females. Several articles have been published in the open literature describing effects on spermatogenesis and developmental/reproduction parameters at high doses. There is also an epidemiology study conducted in farmers exposed to multiple pesticides, which concluded that the miscarriage rate was

increased in parents where the father was exposed to carbaryl. There was no association between the use of carbaryl and preterm delivery, small for gestational age or altered sex ratio measurements. The studies and articles are summarized below.

870.3800 Reproduction and Fertility Effects - Rat

In a two-generation reproduction study (MRID 45448101), carbaryl (99.1% a.i, Lot No. E1208008) was given in the diet to groups of 30 male and 30 female F_0 and F_1 rats (CD®[SD] IGS BR (Sprague-Dawley)) at concentrations of 0, 75, 300, or 1500 ppm. The dietary concentrations corresponded to doses of 4.67, 31.34, and 92.43 mg/kg/day for F_0 males; 0, 5.56, 36.32, and 110.78 mg/kg/day for F_0 females; 0, 5.79, 23.49, and 124.33 mg/kg/day for F_1 males; and 0, 6.41, 26.91, and 135.54 mg/kg/day for F_1 females averaged over the premating period. Each group received treated or control diet continuously for 70 days prior to mating and during mating, gestation, and lactation of one litter per generation. F_1 pups selected to parent the F_2 generation were weaned onto the same food as their parents. Parental males were sacrificed after delivery of their litters and parental females were sacrificed after weaning of their litters.

No treatment-related deaths, clinical signs, organ weight changes, gross lesions, or microscopic lesions were observed in adult rats of either generation. No treatment-related effects were observed on body weights, weight gain, feed consumption, or food efficiency in 75- or 300-ppm group F_0 or F_1 male or female rats at any time during the study including the gestation and lactation periods of the females. F_0 and F_1 male and female rats fed the 1500-ppm diet weighed significantly ($p<0.01$ or <0.05) less and gained less weight during the premating period. The F_0 males weighed 5-6% less than controls during premating, gained 14-23% less weight during three weekly intervals up to day 45, and gained 9% less weight over the entire premating period; they also gained 8% less weight than controls over the mating/postmating period. The F_1 males weighed 10-19% less than controls during the entire study, gained 16% and 11% less weight during the first two weekly intervals, and gained 8% less weight than controls averaged over the entire premating period. The F_0 females weighed 4-5% less than controls during the first 42 days of premating, gained 27% less weight during the first week, and 7% (N.S.) less averaged over the entire premating period. The F_1 females weighed 8-22% less than controls throughout premating and gained 9% less weight during the first week; weight gain for the remaining weekly intervals and for the entire premating period was similar to that of controls. Food consumption and food efficiency for F_0 and F_1 rats followed patterns similar to that of body weight and weight gain; the largest difference between the 1500-ppm groups and controls occurred during the early part of the premating period. When averaged over the entire premating period, F_0 and F_1 males consumed 6-7% less food than control and had food efficiency values similar to those of the controls. Feed consumption and food efficiency for the F_0 females were similar to those of the control group, whereas F_1 females consumed 9% ($p<0.01$) less feed and had a food efficiency value 10% ($p<0.01$) greater than that of controls. F_0 and F_1 females in the 1500 ppm group weighed less and gained less weight than controls during gestation, with the effect being greater in the F_1 females. During lactation weight gain was markedly reduced in F_1 females during the first 4 days, but was greater than that of controls averaged over the entire lactation period.

The lowest-observed-effect level (LOAEL) for parental systemic toxicity is 1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females) based on decreased body weight,

weight gain, and feed consumption. The no-observed-adverse-effect (NOAEL) level is 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females).

No treatment-related effects were observed on the estrous cycle of either F_0 or F_1 females at any dose level or on percent motile sperm, sperm count, percent progressively motile sperm, epididymal sperm count, spermatid head count, daily sperm production, or efficiency of daily sperm production in F_0 or F_1 males at any dose level. There was a dose-related increase in the percentage of abnormal sperm in the treated males but no statistical significance at any dose level. No treatment-related gross or microscopic effects were observed in male or female rats of either generation. No treatment-related effects were observed on any parameter of reproductive performance including, mating and fertility indexes, gestation index, pregnancy index, precoital duration, gestation length, or number of females producing live litters.

The LOAEL for reproductive toxicity could not be established because no effects were observed at any dose level; therefore, the NOAEL is ≥1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females).

No treatment-related effects were observed on implantation sites/litter, number of live pups born/litter, number of dead pups born/litter, live birth index, sex ratio, clinical signs, or organ weight or necropsy findings in pups surviving to 21 days. Pup survival was decreased at 300 and 1500 ppm for both generations. Increased number of deaths in the F_2 generation males and females resulted in an 18-19% decrease in mean litter size on postnatal day 4 ($p<0.01$ or <0.05) and decreased viability and lactation indexes at 1500 ppm. A large number of pups that died had no milk in their stomachs. In addition, pup weight/litter and pup weight gain in the 1500-ppm group pups were reduced for both generations starting with postnatal day 4 (11-15% for F_1 and 13-23% for F_2 pups); body weight gain was reduced throughout lactation with the greatest effect occurring during the first 7 days for F_1 pups and the first 14 days for F_2 pups. Sexual maturation was delayed in 1500-ppm group F_1 offspring as evidenced by delayed balanopreputial separation in the males (+2.1 days) and vaginal patency in the females (+1.4 days). The differences remained statistically significant after adjustment for body weight decreases. Anogenital distance was significantly reduced in F_2 male pups in the 1500-ppm group, but not when the distance was adjusted for body weight.

The LOAEL for offspring toxicity was 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females) based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival. The NOAEL is 75 ppm (4.67-5.79 mg/kg/day for males and 5.56-6.41 mg/kg/day for females).

This study is **Acceptable/Guideline** and satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800; OECD 416) in the rat.

Literature Articles

In a 1996 study in the open literature, carbaryl was administered to four groups of 6 young and 6 adult

Druckery albino rats per group at doses of 0, 25, 50 or 100 mg/kg/day for 60 days.[1] Body weight was recorded at initiation and completion of the study. On the 61st day, the animals were sacrificed and the testes, epididymides, seminal vesicles, ventral prostrate and coagulating glands were weighed. Epididymal sperm were used for sperm counts and examination of motility and morphology. No overt toxicity or mortality was observed. There were dose-related effects on body weight for the 50 and 100 mg/kg/day groups. The absolute weights of the testes, epididymides, seminal vesicle, ventral prostrate and coagulating glands were significantly decreased at 100 mg/kg/day for young rats. The relative organ weights were not affected at any doses. The organ weights were not affected in adult animals. Young rats receiving carbaryl 50 mg/kg/day had a 24.4% and 25% decrease in sperm motility and sperm count, respectively; the changes at 100 mg/kg/day were 42.9% and 37.5%, respectively. Adults receiving the 50 mg/kg/day dose had a 15.1% and 12.5% reduction in sperm motility and count, respectively; the changes at 100 mg/kg/day were 26.4% and 25%, respectively. The percentage of young rats with abnormal sperm was 19.8% and 33.7% at 50 and 100 mg/kg/day, respectively. In adults, the percentages were 16.1% and 23.1% for the respective doses.

In another study from this laboratory, three groups of 8 male Wistar rats per group were administered carbaryl by gavage at doses of 0, 50 or 100 mg/kg/day for 90 days.[2] Body weight was measured periodically throughout the study. On the 91st day, the animals were sacrificed and the male reproductive glands were weighed. One testis from each animal was preserved for histopathology and the other was homogenized for testicular enzyme assay. Epididymal sperm were used for sperm counts and examination of motility and morphology. No clinical signs of toxicity were observed, except for lethargy. Body weights were decreased in the 100 mg/kg/day group after 60 days. There were no changes in the weights of reproductive organs. There were significant changes in the testicular enzymes of the 100 mg/kg/day group: decreases in SDH and G6PDH and increases in GGT and LDH. At both doses, there were significant decreases in the total epididymal sperm count, percent sperm motility and increases in the percent with morphological abnormalities in head, neck and tail. At 50 mg/kg/day, the testes had slight to moderate congestion and edema. A few tubules showed moderately depressed spermatogenesis and loss of sperm. There was moderate atrophy of seminiferous tubules with prominent interstitial spaces in the center of the testes, but the Leydig cells were intact. At 100 mg/kg/day, there were increases in the intensity of congestion and the edematous reaction was seen both peripherally and in the central region. Most of the seminiferous tubules had disturbed spermatogenesis as well as accumulations of cellular masses in their lumens.

In a study conducted at EPA's Health Effects Research Laboratory, 16 pregnant Fischer 344 rats were administered carbaryl by gavage on gestation days (GD) 6-19 at doses of 78 or 104 mg/kg/day; 21

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[1] Pant N, Shankar R, Srivastava SP (1996). Spermatotoxic effects of carbaryl in rats. Human Exp Toxicol 15(9); 736-38.

[2] Pant N, Srivastava SC, Prasad AK, Shankar R, Srivastava SP (1995). Effects of Carbaryl on the Rat's Male Reproductive System. Vet Human Toxicol 37(5): 421-425.

control animals were used.[3] The high dose, selected to produce overt maternal toxicity, was based on the results of a 14-day repeated dose study in nonpregnant female rats. The low dose was 75% of the high dose. Maternal body weights were determined on GD 6, 8, 10, 13, 16 and 20. All rats were examined periodically for clinical signs of toxicity. Pups in each litter were examined and counted on postnatal day (PD) 1, 3, and 6 and weighed collectively on PD 1 and 6. After the final litter examination, the dams were killed and uterine implantation sites counted. Females that did not deliver by GD 24 were killed and their uteri examined for pregnancy status. Clinical signs of toxicity observed in the dams included tremors, motor depression, and lacrimation, usually during the first three days of treatment. Jaw clonus was observed throughout the treatment period. (The article does not indicate if clinical signs were observed at both doses.) Marked weight loss was observed early in treatment. Over the entire treatment period, carbaryl produced extrauterine weight loss at the high dose and reduced weight gains at the low dose. There was increased prenatal mortality at the high dose; this effect was attributed to two (15%) fully resorbed litters in this group. In addition, high dose pup weights were significantly reduced on PD 1. The PD-1 pup weights in the low dose and the PD 6 pup weights in both carbaryl-exposed groups were also significantly reduced, but only when analyzed using the number of live pups on PD 1 as the covariate.

In a recent epidemiology study, the effects of exposure of male farmers in Ontario, Canada, to agricultural pesticides and pregnancy outcome was investigated.[4] Miscarriage risk was not associated with participation in farm activities for all types of chemical applications, but was increased in combination with reported use of thiocarbamates, carbaryl and unclassified pesticides on the farm (Odds ratio = 1.9, 95% C.I. 1.1-3.1). There was no association between use of carbaryl and preterm delivery, small for gestational age or altered sex ratio measurements.

4.5 Chronic Toxicity

Adequacy of data base for chronic toxicity: The data base for chronic toxicity is considered complete. No additional studies are required at this time. In the chronic toxicity study in dogs, at the lowest dose tested, plasma ChEI in females and brain ChEI in males were observed. In a 5-week study to establish the ChEI NOAEL, plasma ChEI was the basis for setting the NOAEL/LOAEL.

870.4100b Chronic Toxicity - Dog

In a chronic toxicity study (MRID No. 40166701), Carbaryl (99%) was administered in the diet to 6 beagle dogs/sex/group at doses of 0, 125, 400 or 1250 ppm for one year. Nominal doses were 3.1, 10 and 31.3 mg/kg/day.

There were no deaths during the study. With the 1250 ppm females, there was an increased incidence of clinical signs of toxicity, including emesis, lacrimation, salivation and tremors. Mean body weight gain

[3] Narotsky MG, Kavlock RJ (1995). A Multidisciplinary Approach to Toxicological Screening: II. Developmental Toxicity. Journal of Toxicology and Environmental Health 45:145-171.

[4] Savitz DA, Arbuckle T, Kaczor D, Curtis KM (1997). Male Pesticide Exposure and Pregnancy Outcome. Am J Epidemiol 146(12):1025-36.

was decreased (50%) in the 1250 ppm females for weeks 0-6. Mean food consumption was decreased (16-24%, not statistically significant) in the 1250 ppm females at multiple time periods during the study. No treatment-related ophthalmoscopic changes were observed. There was a statistically significant increase in white blood cell and segmented neutrophil counts at some of the testing intervals for the 1250 ppm group males. Albumin levels were significantly decreased (9-11%) at all of the testing periods in the 1250 ppm females. Plasma cholinesterase (ChE) levels in males were significantly decreased in the 400 ppm (30-36%↓) and 1250 ppm (58-66%↓) groups at all testing intervals (weeks 5, 13, 26 and 52). Plasma ChE levels in females were significantly decreased at most intervals in the 125 ppm group (12-23%↓), 400 ppm group (9-31%↓) and 1250 ppm group (47-60↓). RBC ChE levels in males were significantly decreased in the 400 ppm group (23-28%↓at weeks 5 and 13) and 1250 ppm group (46-56%↓ for all intervals). RBC ChE levels in females were significantly decreased in the 400 ppm group (29-34% ↓at weeks 5, 13 and 26) and 1250 ppm (29-38% ↓for all intervals). Brain ChE in males was not statistically significantly decreased but biologically decreased in the 400 ppm group (32%↓) and 1250 ppm group (25%↓). Brain ChE in females was significantly decreased (20-36% ↓) in all the groups. No treatment-related effects were seen in urinalysis parameters.

At necropsy, there was a statistically significant increase in the absolute weight of the liver/gall bladder in the 1250 ppm group males. Relative and liver-to-brain weights were also increased but not significantly. There was a dose-related decrease in the absolute, relative and organ-to-brain weights of the pituitary in males, although none of the changes was statistically significant. There was also a significant decrease in the relative weight of the thyroid in this group. However, since there were no accompanying microscopic changes in these organs, the toxicological significance of these organ weight effects is questionable.

The LOAEL for systemic toxicity was 1250 ppm (31.3 mg/kg/day) based on an increased incidence of clinical signs (females), decreased body weight and food consumption (females) and alterations in clinical pathology parameters (both sexes); NOAEL was 400 ppm (10 mg/kg/day).

The LOAEL for plasma cholinesterase inhibition was 125 ppm (3.1 mg/kg/day) for females; a NOAEL was not established. The LOAEL for plasma cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males; the NOAEL was 125 ppm (3.1 mg/kg/day).

The LOAEL for RBC cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males and females; the NOAEL was 125 ppm (3.1 mg/kg/day).

The LOAEL for brain cholinesterase inhibition was 125 ppm (3.1 mg/kg/day) for females; a NOAEL was not established. The LOAEL for brain cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males; the NOAEL was 125 ppm (3.1 mg/kg/day).

In a five-week study (MRID # 42022801), Carbaryl (99.3% a.i.) was administered in the diet to six beagles/sex/group at doses of 0, 20, 45 or 125 ppm. Actual mg/kg/day doses for males were 0, 0.59, 1.43 and 3.83 mg/kg/day, respectively; doses for females were 0, 0.64, 1.54 and 4.11 mg/kg/day, respectively. The following parameters were measured: clinical observations, body weights, food consumption, ophthalmoscopic examinations, plasma and RBC cholinesterase (at days -11, -8 and -5 pretest and then days 14 and 32 of the study), brain cholinesterase (at termination) and gross necropsies.

This study was conducted to complete the information needed to satisfy the chronic toxicity study requirement in nonrodent species.

There were no deaths or treatment-related clinical signs of toxicity. There were no treatment-related effects on body weights, food consumption or ophthalmoscopic examinations. In males, there was a statistically and biologically significant decrease in plasma cholinesterase for the 125 ppm (22%↓) group.

The LOAEL for systemic toxicity and for RBC and brain cholinesterase inhibition was >125 ppm (males: 3.83 mg/kg/day; females: 4.11 mg/kg/day); the NOAEL was ≥ 125 ppm.

The LOAEL for plasma cholinesterase inhibition for males was 125 ppm; the NOAEL was 45 ppm (1.43 mg/kg/day). The LOAEL for cholinesterase inhibition for females was >125 ppm; the NOAEL was ≥ 125 ppm.

Together, these studies are **acceptable** and **satisfy** the guideline requirements for a chronic toxicity study in a nonrodent species (83-1).

4.6 Carcinogenicity

Adequacy of data base for Carcinogenicity: The data base for carcinogenicity is considered complete. No additional studies are required at this time. In both the rat combined chronic toxicity/carcinogenicity study and the mouse carcinogenicity study, there was an increase incidence of tumors, including kidney, liver and vascular tumors, in the treated groups. However, the highest dose in both studies was considered excessive based on evidence of severe toxicity. In addition to the required carcinogenicity studies in mice and rats, the registrant submitted a special study in genetically modified mice. Carbaryl was administered in the diet to heterozygous p53-deficient (knockout) male mice at concentrations of up to 4000 ppm (716.6 mg/kg/day) for six months. There was no evidence of neoplastic or preneoplastic changes in the vascular tissues of any organ. A model validation study demonstrated that vascular tumors occur in heterozygous p53 deficient mice within six months of administration of a known genotoxic carcinogen (urethane). **The CARC (11/7/01) considered all the available toxicity data and concluded that the malignant vascular tumors (hemangiosarcomas) in male mice occurred at doses which were adequate and not excessive. In females these tumors occurred only at the highest dose which was excessively toxic. Nevertheless, the findings in female mice were supportive of vascular tumors in male mice.** The CARC classified carbaryl as "Likely to be carcinogenic in humans" based on an increased incidence of hemangiosarcomas in male mice at all doses tested (100, 1000 and 8000 ppm). The Q_1*, based on the CD-1 mouse dietary study with ¾ Interspecies Scaling Factor, is 8.75×10^{-4} $(mg/kg/day)^{-1}$ in human equivalents.

870.4200b Carcinogenicity (feeding) - Mouse

In a carcinogenicity study (MRID No. 42786901), 80 CD-1® mice/sex/group were administered technical Carbaryl (99.3% a.i.) in the diet at dosages of either 0, 100, 1000 or 8000 ppm for 104 weeks (males: 0, 14.73, 145.99 and 1248.93 mg/kg/day; females: 0, 18.11, 180.86 and 1440.62 mg/kg/day,

respectively.) Four males in the 8000 ppm group died during the first week of treatment; the cause of death was not determined. Survival rates were not affected by treatment.

Animals in the 8000 ppm group, especially the females, developed clinical signs of toxicity, including hunched posture, thin and languid appearance, squinted and opaque eyes, urine stains, redness to various body areas, rough hair coat, soft feces and low body temperature. Mean body weights were statistically significantly decreased for the 8000 ppm males and females for the majority of the study (males 9-13%; females 5-14%). Mean body weight gain for the 8000 ppm males and females was decreased throughout the study (males 23-38%; females 10-32%). Mean food consumption was statistically significantly decreased in the 8000 ppm females (7-10%). Hematology parameters, including RBC, hemoglobin and hematocrit, were statistically significantly decreased in the 8000 ppm females at week 53 and 8000 ppm group males at week 105. Total leukocyte count and counts of lymphocytes and eosinophils were significantly increased in the 8000 ppm group females at week 53. Platelet counts were significantly increased in this group at week 105.

RBC cholinesterase (ChE) was statistically significantly decreased in the 1000 ppm (23%↓) and 8000 ppm (30% ↓) group males at week 53. RBC ChE was decreased in the 8000 ppm group females (24% ↓) at week 105, although the change was not statistically significant. Brain ChE was statistically significantly decreased in the 1000 and 8000 ppm group males at both weeks 53 and 105 (13-18% ↓ for the 1000 ppm group; 40-57% ↓ for the 8000 ppm group) and in the 8000 ppm females (34-47% ↓). Brain ChE was also significantly decreased (13% ↓) in the 1000 ppm group females at week 53. However, the percentage decreases from the control level were less than 20% for the 1000 ppm group males and females at both weeks 53 and 105. Therefore, the biological significance of these findings is questionable. Plasma ChE values were not affected by treatment.

There were no treatment-related macroscopic effects at the week 53 sacrifice, however at the week 105 sacrifice the incidence of opaque eyes was increased in the 8000 ppm group (males: 1/37 controls vs. 4/30; females: 2/34 controls vs. 16/32). The most consistent organ weight changes at both necropsies were increased relative liver and kidney weights. On microscopic examination, there was an increased incidence of chronic progressive nephropathy in the 1000 ppm males and 8000 ppm males and females at the interim sacrifice. The severity of extramedullary hematopoiesis and pigment in the spleen in the 8000 ppm males and females was increased at the interim sacrifice. There was a dose-related increased incidence of intracytoplasmic protein-like droplets in the urinary bladder in the 1000 and 8000 ppm group males and females at the terminal and unscheduled sacrifices. The incidence of animals with cataracts was increased, but not dose-related, in the 8000 ppm group males and females.

The study demonstrated that Carbaryl is carcinogenic in mice at doses of 100 ppm (14.73 mg/kg/day) and higher in males and 8000 ppm (1440.62 mg/kg/day) in females. There was an increased incidence of vascular neoplasms (hemangiomas and hemangiosarcomas) in all treated males and in the 8000 ppm group females at the terminal and unscheduled necropsies but not at week 53. Considering all animals, there was an increased incidence of adenomas, multiple adenomas and carcinomas of the kidney in the 8000 ppm group males. The incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was increased in the 8000 ppm group females. The HED CPRC concluded that the 8000 ppm dose was excessive based on the significantly decreased body weight gain in males (33%) and females (19%) during week 13, a significant decrease in RBC and brain cholinesterase activity,

clinical signs of toxicity and histopathological changes in the bladder, kidneys and spleen in both sexes.

The systemic LOAEL was 1000 ppm (M: 145.99 mg/kg/day; F: 180.86 mg/kg/day) based on an increased incidence of intracytoplasmic droplets in the superficial epithelial cells of the urinary bladder in males and females and chronic progressive nephropathy in males. The systemic NOAEL was 100 ppm (M:14.73 mg/kg/day; F: 18.11 mg/kg/day).

The RBC cholinesterase inhibition LOAEL in males was 1000 ppm (23% ↓ at week 53); the NOAEL was 100 ppm. The RBC cholinesterase inhibition LOAEL in females was 8000 ppm (24%↓at week 105); the NOAEL was 1000 ppm.

The plasma cholinesterase inhibition LOAEL was >8000 ppm (M: 1248.93 mg/kg/day; F: 1440.62 mg/kg/day); the NOAEL was ≥ 8000 ppm.

The brain cholinesterase inhibition LOAEL for males and females was 8000 ppm (M: 40-57%↓; F: 34-47%↓); the NOAEL was 1000 ppm.

This study is classified as **Acceptable** and **satisfies** the guidelines for a carcinogenicity study in mice (§83-2).

870.4300 Combined Chronic Toxicity/Carcinogenicity Study - rat

In a combined carcinogenicity/chronic toxicity study (MRID No. 42918801), 70 Sprague-Dawley Crl:CD®BR rats/sex/group were administered technical Carbaryl (99% a.i.) in the diet at dosages of either 0, 250, 1500 or 7500 ppm for 104 weeks (males: 0, 10.0, 60.2 and 349.5 mg/kg/day; females: 0, 12.6, 78.6 and 484.6 mg/kg/day). An additional 10 animals/sex/dose were administered the same doses and were sacrificed after 53 weeks. Another 10 animals/sex from the control and high dose group animals were sacrificed at week 57 after switching the diet of the high dose animals to control feed for weeks 53-57 of the study.

There was no treatment-related effect on survival. There was an increased incidence of clinical signs of toxicity, including hunched posture, thin appearance, chromodacryorrhea and urine stains in the 7500 ppm group males. There was an increased incidence of alopecia and urine strains in the 7500 ppm group females.

Statistically significant decreases in mean body weight were observed in the 7500 ppm males (24-35%) and females (24-45%) and the 1500 ppm females (4-12%). Mean body weight gain over the course of the study was decreased in the 7500 ppm males (53%) and females (69%). There was a 18% decrease in body weight gain in the 1500 ppm females for the week 0-104 period only. Food consumption in the 7500 ppm group males and females was decreased (4-16% in males; 11-21% in females) during the study. In the recovery group, rebound in food consumption and body weight gain was seen, but mean body weight was still decreased 23% for both the 7500 ppm males and females at week 57.

There was an increased incidence of unilateral and bilateral cataracts in the 7500 ppm males and females. A consistent decrease in WBC and lymphocyte count in the 7500 ppm males and females was seen.

Alterations in clinical chemistry in the 7500 ppm males and females included significant increases in cholesterol and BUN and significant decreases in AST, ALT and CPK. Plasma cholinesterase was decreased in the 7500 ppm males (27-42%) and females (46-57%) at all of the testing intervals (weeks 27, 53, 79 and 105), however all of the changes were not statistically significant. RBC cholinesterase was decreased in the 7500 males (19-37%) and females (25-38%) and in the 1500 ppm males (10-23%) and females (12-26%) at most of the testing intervals. At weeks 53 and 105, brain cholinesterase was statistically significantly decreased in the 7500 ppm males (8-28%) and females (22-31%). In the recovery group, cholinesterase values had returned to normal levels by week 56.

There was a slightly increased incidence of erythrocytes in the urine of the 7500 ppm males and occult blood in the 7500 ppm males and females. An increased incidence of dark urine in the 1500 ppm females and in the 7500 ppm males and females was also found.

There were no treatment-related macroscopic findings at the week 53 and 57 necropsies. At the week 105 necropsy, the macroscopic findings at an increased incidence in the 7500 ppm males and females, which were also associated with microscopic changes, included pale areas in the lungs and liver and urinary bladder masses. A decreased absolute weight and an increased relative weight of the kidneys, lungs, spleen and liver were found in the 7500 ppm males and females. At the week 53 necropsy, there were slight increases in the incidence of microscopic changes in the kidney and liver of the 7500 ppm males and females. At the week 105 necropsy, there was a wide variety of changes in multiple organs of males and females in the 7500 ppm group. In the liver, there was an increased incidence in the following: hepatocytic hypertrophy in males and females; and eosinophilic foci and pigment in females. In the urinary bladder, there was an increased incidence of transitional cell hyperplasia, squamous metaplasia, high mitotic index and atypia in males and females. In the lung, there was an increased incidence of focal pneumonitis and foamy macrophages in males and females. In the kidney, there was an increased incidence of transitional cell hyperplasia in males. In the thyroid, there was an increased incidence of follicular cell hypertrophy in males and females. Degeneration of the sciatic nerve and skeletal muscle was observed at an increased incidence in males and females.

The study demonstrated that Carbaryl is carcinogenic in male and female rats at 7500 ppm. There was an increased incidence of liver adenomas in females. In the bladder, there was an increased incidence of benign transitional cell papilloma and transitional cell carcinomas in males and females. One transitional cell carcinoma was also observed in the kidney of a male rat. In the thyroid, the incidence of benign follicular cell adenomas was increased in males; one follicular cell carcinoma was also seen in a male.

The HED CPRC evaluated the toxicity data on Carbaryl and considered 7500 ppm to be an excessive dose based on the following findings: 1) changes in body weight gain during week 13 for males and females by 40% and 52%, respectively, as compared to controls; 2) decreased food efficiency; 3) alterations in hematology and clinical chemistry; and 4) decreases in plasma, RBC and brain cholinesterase at weeks 53 and 105.

The systemic LOAEL was 1500 ppm (78.6 mg/kg/day) in females based on decreased body weight and body weight gain; the NOAEL was 250 ppm (12.6 mg/kg/day). The systemic LOAEL was 7500 ppm (349.5 mg/kg/day) in males based on an increased incidence of clinical signs of toxicity, decreases in

body weight, body weight gain and food consumption, an increased incidence of cataracts, alterations in clinical pathology parameters, organ weight changes, and an increased incidence of nonneoplastic microscopic changes. The systemic NOAEL was 1500 ppm (60.2 mg/kg/day) in males.

The LOAEL for plasma cholinesterase inhibition was 7500 ppm in males (27-47% decrease) and females (46-57% decrease); the NOAEL was 1500 ppm.

The LOAEL for RBC cholinesterase inhibition was 1500 ppm in males (10-23% decrease) and females (12-26% decrease); the NOAEL was 250 ppm.

The LOAEL for brain cholinesterase inhibition was 7500 ppm in males (8-28% decrease) and females (22-31% decrease); the NOAEL was 1500 ppm.

This study is classified as **Acceptable** and **satisfies** the guidelines for a combined carcinogenicity/chronic toxicity feeding study in rats (83-5).

Carcinogenicity and Other Studies in p53 Knockout Mice

In a special, non-guideline study (MRID 45281801, 45281802, 45236603), heterozygous p53-deficient (knockout) male mice (20/group) were administered carbaryl in the diet at concentrations of 0, 10, 30, 100, 300, 1000 and 4000 ppm (approximately 0, 1.8, 5.2, 17.5, 51.2, 164.5 and 716.6 mg/kg/day, respectively) for six months. The doses selected for this study were based on two 28-day studies (MRID 45236603) in wild-type mice in which body weight decreases were observed at 4000 and 8000 ppm concentrations of carbaryl in the diet. A validation study (MRID 45281802) demonstrated that vascular tumors occur in heterozygous p53-deficient mice within 6 months of administration of a known genotoxic carcinogen (urethane). These studies were conducted to demonstrate that carbaryl is a non-genotoxic carcinogen. In the standard mouse carcinogenicity study (MRID 42786901) at dietary concentrations of 0, 100, 1000 or 8000 ppm, there was an increased incidence of vascular neoplasms (hemangiomas and hemangiosarcomas) in all treated males and in the 8000 ppm group females. There was an increased incidence of adenomas, multiple adenomas and carcinomas of the kidney in the 8000 ppm group males. The incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was increased in the 8000 ppm group females. At meetings on October 27 and December 8, 1993, the HED Cancer Peer Review Committee concluded that the 8000 ppm dose was excessive. Therefore, the relevance of tumors at this dose was questionable.

In the p53 knockout mouse study with carbaryl, there was a slight decrease in body weight and food consumption in the 4000 ppm group. No other treatment-related effects were observed, except globular deposits in the urinary bladder were observed in a high proportion of the mice treated at 100 ppm of carbaryl and above with a dose-related increase in incidence and severity. There was no evidence of local irritation or hypertrophy of the bladder epithelium. There was no evidence of neoplastic or preneoplastic changes in the vascular tissue of any organs examined.

The study is classified **Acceptable (non-guideline)**. This is a special study not submitted to fulfill a data requirement.

4.7 Mutagenicity

Adequacy of data base for Mutagenicity: The data base for mutagenicity is considered complete and no additional studies are required at this time.A recent review of the data from the submitted studies and the published literature were in general agreement and showed that carbaryl was clastogenic *in vitro.* The wide variety of induced aberrations (both simple and complex) was consistent between the submitted study and the open literature. However, there were inconsistencies relative to the requirement for S9 activation.

Nevertheless, the two *in vivo* studies for micronuclei induction or chromosome aberrations were negative. Similarly, the 6-month p53 knockout transgenic mouse bioassay (see Section 4.6) was negative up to a high level (4000 ppm, ≈720 mg/kg/day) that approached the limit dose for a mouse carcinogenicity assay. Carbaryl was also negative for DNA binding in the livers of mice treated with 8000 ppm for 2 weeks but the study was considered to be of limited sensitivity by the CARC Metabolism Subgroup. The same Subgroup identified epoxide intermediates of carbaryl which were found to be conjugated to glucuronide, "rapidly metabolized and excreted as any endogenous epoxide would be".

Overall, these findings indicate that carbaryl produces epoxides and its DNA reactivity is manifested as chromosomal aberrations in cultured mammalian cells. Other *in vitro* studies indicate carbaryl's effects on karyokinesis and cytokinesis, as well as stress genes associated with oxidative damage. Based on these considerations, it was concluded that there is a concern for mutagenicity, which is somewhat lessened because of the lack of an effect in *in vivo* mutagenicity studies.

GENE MUTATIONS

Mutagenicity - Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay (Ames test)

In a Salmonella/mammalian activation gene mutation assay (MRID 41370303), carbaryl technical (99.3%) was initially evaluated in the Salmonella typhimurium/microsome mutagenicity assay over a concentration range of 5 to 1000 µg/plate. The test material was not mutagenic, however the highest assayed dose was cytotoxic in S. typhimurium strains TA98 and TA100, but not in strains TA1535, TA1537, or TA1538. Accordingly, the assay was repeated with six concentrations (10 to 2000 µg/plate +/-S9). Results from the repeat assay indicated that 2000 µg/plate +/-S9 was cytotoxic in strains TA98 and TA100, and the remaining doses were not mutagenic. It is concluded, therefore, that carbaryl technical was assayed to an appropriately high concentration with no evidence of mutagenicity in a well-conducted study. The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements (§84-2) of bacterial reverse mutation test.

Mutagenicity - Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells

In a mammalian cells in culture gene mutation assay in Chinese Hamster Ovary (CHO) Cells (MRIDs 41370302, 41420201), carbaryl technical (99.3%) was evaluated in two nonactivated and three

S-9 activated Chinese hamster ovary (CHO) cell forward mutation assays. The findings from both nonactivated assays were in good agreement and indicated that over a concentration range of 1 to 300 µg/mL, the test material did not induce a mutagenic response. Doses ≥200 µg/mL were severely cytotoxic (<10% cell survival), and <50% of the cells survived exposure to ≥50 µg/mL. Carbaryl was less cytotoxic in the presence of S9 activation as indicated by increased survival at comparable levels in the preliminary cytotoxicity test (e.g., 29.5% survival at 62.5 µg/mL -S9 as compared with 95.7% survival at 62.5 µg/mL +S9) and the initial mutation assay (e.g., 18.1% survival at 100 µg/mL -S9 as compared with 46.8% at 100 µg/mL +S9). There was no definitive evidence of increased mutation frequencies (MFs) in this trial. The second S9-activated trial was aborted because of excessive cytotoxicity at test material levels of ≥10 µg/mL. Results from the third S9-activated trial (dose range: 1 to 80 µg/mL) showed severe cytotoxic effects at levels ≥60 µg/mL; no evidence of mutagenic effect was seen at the remaining doses.

The results of the assays provide no clear indication of a mutagenic response, however, the study does not fully support a negative conclusion. The conflicting cytotoxicity data for the S9-activated assays provide no assurance that the final S9-activated mutation assay was conducted over an appropriate dose range. The study is classified as **unacceptable/guideline** and **does not satisfy** the guideline requirements (§84-2) for an *in vitro* mammalian cell gene mutation test.

CHROMOSOME ABERRATIONS

Mutagenicity - Mammalian Cells in Culture Cytogenetic Assay

Carbaryl (technical) was assayed for clastogenic effects in both the presence and absence of S9 activation using Chinese hamster ovary (CHO) cells (MRID 41370304). Because of severe cell cycle delay, which was more pronounced without S9 activation, a 20-hour cell harvest was selected to evaluate seven nonactivated doses ranging from 5 to 100 µg/mL. In the presence of S9 activation, cells exposed to carbaryl at doses of 25, 50, 75, 100, 150, 200, 250, and 300 µg/mL were harvested 30 hours post treatment. Results indicated that the nonactivated test material was more cytotoxic than the S9-activated test material (*i.e.*, few metaphases were recovered at 75 and 100 µg/mL , and moderate to slight cytotoxic effects were seen at doses ≥10.0 µg/mL). With the exception of a single rare complex aberration (quadriradial) scored at the 50.0-µg/mL dose level, there was no evidence of a clastogenic effect. By contrast, in the S9-activated assays, all scored doses (150, 200, 250, and 300 µg/mL) at both harvest times induced significant ($p \leq 0.01$) increases in the percentage of cells with aberrations. The majority of S9-activated doses (both harvests) also induced significant ($p \leq 0.01$) increases in the percentage of cells with >1 aberration. At both the 20- and 30-hour harvest times, cytotoxicity (*i.e.*, reduced monolayers, dead cells, and/or reduced mitotic cells) were observed at levels ≥200 µg/mL. Induced structural damage included simple (*i.e.*, chromatid and chromosome breaks) and complex aberrations (*i.e.*, triadials, quadriradials, complex rearrangements, dicentrics and rings). The data show little or no dose responsiveness and the lowest reactive level of carbaryl was not determined. It was concluded, however, that the study was technically sound and, therefore, **acceptable/guideline**. The study **satisfies** the Guideline requirements (§84-2) for an *in vitro* mammalian cell chromosomal aberration test.

Mutagenicity - Mouse Micronucleus Test

In a mouse micronucleus assay (MRID No: 44069301), groups of five male and five female CD-1 mice received single oral gavage administrations of 50, 100 or 200 mg/kg carbaryl (99.9%) once daily for 2 days. Based on analytical determinations, average daily doses were ≈34, 79 or 180 mg/kg. Mice were sacrificed at 24 and 48 hours postadministration of the second dose and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs). The test material was delivered as suspensions prepared in 0.5% carboxymethyl cellulose.

The minimal toxicity (i.e., lethargy which lasted for 2 hours) in the absence of cytotoxicity to the target cells does not support the testing of the maximum tolerated dose (MTD). The positive control induced the expected high yield of MPEs in males and females. Carbaryl did not induce a clastogenic or aneugenic effect in either sex at any dose or sacrifice time. However, there was no convincing evidence that the MTD was achieved**.** The study is classified as **unacceptable/guideline** and **does not satisfy** the guideline requirements(§84-2; OPPTS 870.5385) for *in vivo* cytogenetic mutagenicity data.

OTHER MUTAGENIC EFFECTS

Mutagenicity - UDS Assay

In a UDS Assay in primary rat hepatocytes (MRID 41370301), under the conditions of two independent trials, six doses of carbaryl technical (99.3%) ranging from 0.5 to 25.0 µg/mL in the first assay and six doses ranging from 5.0 to 25.0 µg/mL in the repeat assay did not induce an appreciable increase in the net nuclear grain counts of treated rat hepatocytes. Doses >25.0 µg/mL were severely cytotoxic; reduced cell survival (≈25%) was observed at 25.0 µg/mL in both assays. Although an increase in the percentage of cells with ≥ 6 grains per nucleus was seen in the initial test, the increase was confined to a single dose (10 µg/mL) and was not dose-related or reproducible. The study demonstrated that carbaryl is not genotoxic in this test system at doses of 5.0 to 25.0 µg/mL. The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements (§84-2) for a unscheduled DNA synthesis in mammalian cells in culture.

STUDIES FROM THE OPEN LITERATURE

Studies in the open literature indicate that Carbaryl is not mutagenic in bacteria but produced conflicting results in Chinese hamster V79 gene mutation assays [negative in the study of Onfelt and Klasterska (1984) but weakly positive minus S9 metabolic activation as reported by Ahmed et al. (1977)]. Nonactivated carbaryl induced aneuploidy and sister chromatid exchanges in V79 cells; the addition of S9 or an excess of glutathione eliminated these responses (Onfelt and Klasterska 1983, 1984). In the former study, multiple chromatid exchanges (quadriradials and complex rearrangements) plus chromosome breaks were also induced by 100 mM carbaryl; this effect was largely abolished by the simultaneous addition of S9 or glutathione. There were positive data for DNA damage in a human lymphoblastoid cell line (induction of CYP1A1 genes); carbaryl also activated other stress genes known to be sensitive to oxidative damage (Delescluse *et al.*, 2001). Also, carbaryl caused depolymerization of spindle microtubules and an apparent uncoupling of karyokinesis and cytokinesis in cultured V79 cells (Renglin *et al*, 1988, 1989).

In contrast to the *in vitro* data, carbaryl administered by oral gavage at 1/3 of the LD_{50} (146 mk/kg/day) for 2 consecutive days was negative for micronuclei induction in Swiss albino male mice (Usha Rani et al., 1980). Carbaryl was also negative for the induction of chromosome aberrations in bone marrow cells of Syrian hamsters treated with 1/10, 1/5 and ½, of the LD_{50} and the LD_{50} (Dzwonkowska and Hubner, 1986).

4.8 Neurotoxicity

Adequacy of data base for Neurotoxicity: Available neurotoxicity studies are adequate to satisfy the guideline requirements. There was no evidence of delayed neurotoxicity in hens. In the acute neurotoxicity study in rats, the LOAEL was based on plasma, RBC and brain ChEI; a NOAEL could not be established. In the subchronic neurotoxicity study in rats, clinical signs of toxicity were seen at the same dose as plasma, whole blood, RBC and brain ChEI. There was no evidence of structural neuropathology in these studies. In the developmental neurotoxicity study in rats, clinical signs of toxicity and plasma and brain ChEI were seen in maternal animals at the same dose as changes in brain morphometric measurements in offspring. The HED Hazard Identification Assessment Committee (HIARC) determined that this was evidence of qualitative susceptibility.

870.6100 Delayed Neurotoxicity Study - Hen

In a study by Carpenter *et al* [5], Carbaryl was negative for delayed neuropathy at a dose of 2000 mg/kg, the approximate LD_{50} in hens.

870.6200 Acute Neurotoxicity Screening Battery

In an acute neurotoxicity study (MRID # 43845201-43845204), groups of 12 male and 12 female Sprague-Dawley rats were administered Carbaryl technical grade in 0.5% carboxymethylcellulose / 0.1% Tween 80 at doses of 10, 50, or 125 mg/kg/day. Doses were selected on the basis of results from a benchmark toxicity study (MRID # 43845201) and a "time of peak effects" study (MRID # 43845202). In the benchmark study, clinical signs of toxicity and body weight loss were observed at 50 mg/kg/ and above, and mortality was observed at 500 mg/kg and above. In the time of peak effects study, peak effect for cholinesterase inhibition and functional observational battery changes was determined to be 0.5 to 1.0 hr post-dose. Body weight was mildly but significantly decreased in male rats at the 125 mg/kg dose level, while weight gain was significantly decreased in male and female rats for days 0-7 of the study at 125 mg/kg. Food consumption during week 1 was decreased at the 125 mg/kg dose by 18-20%, in excess of the decrease in body weight gain, supporting a treatment-related effect at the high dose for week 1 of the study. Several measurements from Functional Observational Battery assessment were significantly altered at the 50 and 125 mg/kg dose, including an increased incidence of tremors, ataxic gait, decreased body temperature, and decreased arousal. Salivation incidence was increased at the high dose, as was hindlimb splay. Forelimb and hindlimb grip strength were decreased significantly at the high dose. Significant decreases in total motor activity were observed in male and female rats at all dose levels tested. Significant inhibition of plasma, blood, and brain cholinesterase (30-40%) was also observed in both sexes at the 10, 30 and 90 mg/kg doses. Peak inhibition of cholinesterase occurred during the time of FOB and motor activity measurements. Based on the data in this study, the systemic LEL = 10 mg/kg for male and female rats, based on significant inhibition of red cell, plasma, whole blood, and brain cholinesterase at the 10 mg/kg dose level. The systemic NOAEL < 10 mg/kg for male and female rats. Although significant signs of cholinergic toxicity were observed in this study, there was no definitive evidence of a neurotoxic effect for Carbaryl technical grade in this study. This study is classified as **acceptable** and **satisfies** the guideline requirement for an acute neurotoxicity study (§81-8) in rats.

870.6200 Subchronic Neurotoxicity Screening Battery

In a subchronic neurotoxicity study (MRID 44122601), 12 Crl:CD(SD)BR rats/sex/group were administered technical Carbaryl (99.1%) by gavage at doses of 0, 1, 10 or 30 mg/kg/day for 13 weeks. Cholinesterase (RBC, whole blood, plasma and brain) determinations were done on an additional three groups of five rats/sex/group at Weeks 4, 8 and 13. Neurobehavioral screening, consisting of Functional Observational Battery (FOB) and motor activity evaluations, was performed prior to treatment and during

[5] Carpenter, C.P., Weil, C.S., Palm, P.E. *et al*. Mammalian Toxicity of 1-napthyl-N-methyl carbamate (Sevin Insecticide). J. Agri. Food Chem. 9(1):30-39, 1961.

Weeks 4, 8 and 13. At terminal sacrifice, six animals/sex/dose were anesthetized and perfusion fixed *in situ* for neuropathological evaluation.

There were no deaths during the study. There was an increased incidence of clinical signs of toxicity, including slight and moderate salivation and tremors, in the 30 mg/kg/day males and females. Body weight over the course of the study was statistically significantly decreased in the 30 mg/kg/day males (14%) and females (15%). Body weight gain for these groups was decreased 27% in males and 37% in females, compared to controls. Food consumption was decreased during most of the study for the 30 mg/kg/day males and females. Males and females in the 30 mg/kg/day group had a statistically significant decrease in RBC (M:42-46%; F:52-55%), whole blood (M: 49-51%; F: 59-63%) and plasma cholinesterase values (M: 63-69%; F: 63-69%) at most of the testing periods. Males and females in the 10 mg/kg/day group had a statistically significant decrease in RBC (M: 26-38%; F: 17-24%); whole blood (M: 30-41%; F: 21-26%) and plasma cholinesterase values (M:43-48%; F: 23-30%). There was a statistically significant decrease in brain cholinesterase in males and females in the 10 mg/kg/day (M: 27-61%; F: 20-58%) and 30 mg/kg/day (M: 36-80%; F: 50-73%) groups. For the 1 mg/kg/day males, there were statistically significant decreases in whole blood (13%) at week 13 and for plasma (20%) at week 8. These changes are not considered toxicologically significant since they occurred infrequently and were relatively minor effects.

Multiple qualitative and quantitative FOB parameters were affected in the 10 and 30 mg/kg/day males and females, including the following: slight tremors, gait alterations, pinpoint pupils, increased salivation, reduced extensor thrust, decreased pinna reflex, reduced number of rearings, decreased vocalizations, decreased body temperature and decreased forelimb grip. Reduced number of defecations was observed only at 30 mg/kg/day. There was an occasional alteration at the 1 mg/kg/day dose. At week 8, males had a very slight increase in the incidence of pinpoint pupils (incidence in control, 1, 10 and 30 mg/kg/day groups was 0/12, 1/12, 6/12 and 10/12, respectively). A statistically significant decrease in forelimb grip was observed at week 4 in males (values for control, 1, 10 and 30 mg/kg/day groups were 1060.8, 943.8, 943.8 and 950.0, respectively). The number of defecations was statistically reduced in females at week 13 (mean number of defecations in control, 1, 10 and 30 mg/kg/day groups were 1.4, 0.2, 0.5 and 0.0, respectively). The toxicological significance of these effects in the 1 mg/kg/day group is questionable since the incidence was either low or there was no dose-response relationship.

Motor activity was statistically significantly decreased in the 30 mg/kg/day males at Week 4 and the 30 mg/kg/day females at Weeks 4 and 8.

On necropsy, there was an increased incidence of dark areas in the meninges of the 30 mg/kg/day males; these animals had an increased incidence of hemorrhage on microscopic examination. One female in the 30 mg/kg/day group also had retinal atrophy. There were no differences in brain length or width measurements.

The LOAEL for neurotoxicity was 10.0 mg/kg/day based on an increased incidence of FOB changes; the NOAEL was 1.0 mg/kg/day. The LOAEL for cholinesterase inhibition was 10.0 mg/kg/day based on statistically significant decreases in RBC, whole blood, plasma and brain cholinesterase; the NOAEL was 1.0 mg/kg/day.

The subchronic neurotoxicity study in the rat is classified **acceptable/guideline** and **does satisfy** the guideline requirement for a subchronic neurotoxicity study (OPPTS 870.6200) in the rat.

870.6300 Developmental Neurotoxicity Study

In a developmental neurotoxicity study (MRID # 44393701, 44904204, 45456701, 45456702, 45456703), 26 pregnant female Sprague-Dawley rats/group were administered carbaryl (99.1% a.i.) by gavage from Gestation Day (GD) 6 through Lactation Day (LD) 10 at doses of either 0, 0.1, 1.0 or 10 mg/kg/day. An additional 6 pregnant females/group were dosed at the same levels for the cholinesterase (ChE) phase of the study. ChE measurements were done pre-dosing (GD 6) and post-dosing at time of peak effect (1 hour post-dosing) on GD 6, 15 and 20 and LD 4 and 10. Functional Observational Battery (FOB) measurements were performed at approximately 0.5 and 2 hours post-dosing on the same days as body weight measurements during the dosing period (GD 0, 6, 9, 12, 15, 18 and 20 and LD 4, 7, 11, 13 and 21). Measures of reproductive performance were evaluated. Offspring were examined for body weight, physical development landmarks (tooth eruption and eye opening), FOB assessments (days 4, 7, 11, 13, 17 and 21) and motor activity (days 13, 17 and 21). On LD 11, 1 animal/sex/litter was sacrificed for brain weights; of these, six/sex were randomly selected for neuropathological evaluation. The eyes from all dose groups were examined. After LD 21, 3 animals/sex/litter were separated from the dams and constituted the F1 adult generation. These animals were evaluated for body weight, physical development (vaginal opening and preputial separation), motor activity (day 60), startle habituation response (days 22 and 60), passive avoidance (day 23) and water maze behavior (day 60). After completion of the behavior test period (at approximately 10 weeks of age), 12 animals/sex/group were anesthetized and perfused for post-mortem examination. Tissues from 6 animals/sex of the control and high dose group were processed for neuropathological evaluation and morphometric measurements; the eyes from the low and mid-dose group of all perfused animals were examined.

For the F0 generation animals, there were no carbaryl-associated deaths. No treatment-related clinical signs of toxicity were observed. There was a statistically significant decrease (92%) in body weight gain for females in the 10 mg/kg/day group for the period GD 6-9. Unfortunately, food consumption was not measured during the study. During the FOB measurements, the incidence of females in the 10 mg/kg/day group with decreased pupil size (pinpoint pupils) was increased on all occasions during the dosing period. An increased incidence of dams with slight tremors affecting the head, body and/or limbs was noted on the majority of assessment occasions in the dosing period. There were also occasional occurrences of ataxic gait/overall gait in-capacity which was considered to be of toxicological significance due to other effects upon gait.

For the 10 mg/kg/day group, RBC and whole blood ChE levels were statistically significantly decreased (28% and 32-34%, respectively) on GD 20 and LD 10. Although the plasma ChE levels were not statistically significantly altered, the percentage decreases on GD 20, LD 4 and

LD 10 were 32-39%. Brain ChE levels were statistically significantly decreased (42%). There were no treatment-related effects on gross necropsy findings for the F0 generation animals.

There were no effects observed on maternal performance parameters of pregnancy rate, gestation index, length of gestation, numbers of live pups, dead or malformed pups, implantation scars, sex ratio or post-implantation loss. There was a slight (P>0.05) increase in the number of dead pups in the 10 mg/kg/day group, however the value was within the historical control range for this strain.

For the F1 generation pups, there were no treatment-related effects on pup weight, pup survival indices, developmental landmarks (tooth eruption and eye opening), FOB measurements or motor activity assessments. At sacrifice on LD 11, there were no treatment-related effects on brain weight and gross or microscopic pathology. Significant differences noted in the morphometric measurements included an increase in Line B of the right forebrain and Line F of the left cerebellum in the 10 mg/kg/day males. In the 10 mg/kg/day females, Line F through both the right and left cerebellum were significantly decreased (15% and 22%, respectively).

For the F1 generation adults, there were no treatment-related effects on clinical condition, body weight, physical development (vaginal opening and preputial separation), motor activity, auditory startle response, passive avoidance and water maze measurements. At sacrifice, there were no gross or microscopic neuropathological lesions observed for animals examined in this study that were attributable to treatment with the test article. There was an increased incidence of retinal fold/rosette in the 10 mg/kg/day group (1/12 for control vs. 4/12 for males; 0/12 for control vs. 2/12 for females). The finding was not considered of toxicological significance since the incidence was within the historical control range for males, occurred at a low rate and was not dose-dependent. For the morphometric measurements, there was a significant bilateral decrease in Line A through the forebrain (7.7-9.8%) and a significant increase in Line F through the right cerebellum of the 10 mg/kg/day males. Increases originally noted in 10 mg/kg adult females in Line G, width of the cerebellum, were found to be based on erroneous measurements, and additional measures were submitted. Now, for the 10 mg/kg/day females, there were significant bilateral increases in Line F through the cerebellum (7.4-15%). Measurements of the size of the thickness of lobes and of the granule cell layers of the cerebellum in high dose pups and adults did not differ from those of controls. While additional statistical analyses by the registrant indicated no treatment related effects, HED's additional statisical analyses did indicate treatment related effects.

The maternal toxicity LOAEL was 10 mg/kg/day based on decreased body weight gain, alterations in FOB measurements and RBC, plasma, whole blood and brain cholinesterase inhibition. The maternal NOAEL was 1.0 mg/kg/day.

The developmental neurotoxicity LOAEL was 10 mg/kg/day based on a bilateral decrease in the size of the forebrain (Line A) in adult males (7.7-9.8%); a bilateral decrease in the length of the cerebella (Line F) in female pups (15-22%); and a bilateral increase in the length of the cerebella (Line F) in female adults (7.4-15%).

The developmental NOAEL was 1 mg/kg/day. Morphometric assessment at the mid and low doses could not be conducted due to inadequate tissue storage; however, based on the minimal findings at the LOAEL, it is HED's judgment that effects would be unlikely to occur at 1 mg/kg/day, which is 10% of the LOAEL.

4.9 Metabolism

Adequacy of data base for metabolism: Available metabolism data are adequate to satisfy the guideline requirements and have delineated the metabolic pathway in the rat. Carbaryl was broken down into over 20 metabolites. The major route of elimination was via the urine. No significant tissue accumulation was reported. Additional special studies have been conducted to determine if there are alterations in metabolism at high doses.

870.7485 Metabolism - Rat

In a rat metabolism study (MRID # 43332101), ^{14}C-Carbaryl was administered orally in carboxymethylcellulose or intravenously in sodium phosphate buffer (pH 6.8) to groups (5 sex/dose) of male and female Sprague-Dawley rats at nominal doses of 1 mg/kg (single and repeated low oral doses; intravenous dose) and 50 mg/kg (single high oral dose). Absorption was essentially complete for all dose groups of male and female rats. At 168 hours post-dose, there were negligible percentages of the dose found in any tissue examined. On a µg/g tissue basis, kidney and blood were found to contain the highest concentrations of residual radioactivity, with female rats showing slightly higher values than males. Excretion of carbaryl derived radioactivity was largely through urine, where 88-95% of the dose was recovered for all dose groups. There were no significant dose- or sex-related differences in excretion.

Conjugated metabolites of carbaryl identified in this study included the glucuronic acid conjugate of dihydro-dihydroxy carbaryl (2.2% of the dose), the S(N-acetylcysteine) conjugate of dihydro-hydroxy carbaryl (3.7% of the dose), naphthyl glucuronide (2.0% of the dose), and naphthyl sulfate (6.4% of the dose). Non-conjugated metabolites identified were 1-naphthol, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, 4-hydroxycarbaryl, and N-(hydroxymethyl)-hydroxycarbaryl. These accounted for 14.5%, 12.8%, 8.2%, 6.3%, and 5.7% of the administered dose, respectively. Three new metabolites were identified in this study which were the N-(hydroxymethyl)-hydroxycarbaryl metabolite, hydroxy-desmethylcarbaryl (0.5% of the dose), and the S-(N-acetylcysteinyl)-dihydro-dihydroxycarbaryl conjugate. Based on these data, a metabolic scheme for carbaryl was proposed. This study is classified as **acceptable/guideline** and **satisfies** the data requirements for a metabolism study in rats under Subdivision F guideline §85-1.

Metabolism - Special Study

In a rat metabolism study (MRID No. 44402501), 1-naphthyl-^{14}C-labeled carbaryl (ca 100% a.i.) was administered to 15 month old male Iffa Credo CD (Sprague-Dawley derived) rats (5 animals/group) as a single oral gavage dose of 50 mg/kg (group A) or as a daily oral dose of 2 mg/kg for 7 days following a 83-day dietary administration with non-radioactive carbaryl (25 animals/group) at 0 (group B), 250 (group C), 1500 (group E), or 7500 ppm (group D). This study was designed to "investigate the mechanisms that caused the appearance of an increased incidence of tumors during the final year of a chronic dietary feeding study in the rat at the high dose level of 7500 ppm."

In all dietary dosing regimens, urinary and fecal excretion totaled 96-103% of the administered dose. Most of the radioactivity was eliminated in the urine and feces within 24 hours after dosing. In the

group A, 86% and 11% of the test compound administered was excreted in the urine and feces, respectively, over a 7-day period after a single dose via gavage of radiolabeled carbaryl at 50 mg/kg. In the groups B-E (0, 250, 1500, and 7500 ppm), 3 days after the 7th consecutive administration of radiolabeled carbaryl, 79-89% and 7-10% of the total administered dose (sum of the 7 daily doses) were excreted in the urine and feces, respectively.Tissue distribution study showed that the levels of radioactivity in the tissues of the animals from group A were 0.4% of the administered dose at sacrifice (168 hours after dosing). In groups B-E, the levels of radioactivity in the tissues ranged from 0.4-0.8% of the administered dose 3 days after the 7th dose of radiolabeled carbaryl at 2 mg/kg. This indicates that the potential for bioaccumulation of carbaryl in rats is minimal.

HPLC analysis of carbaryl metabolites in 24-hour urine samples showed a total of 23 components. Four components identified by LC/MS technique were as follows: UMET/8 (trans-5,6-dihydro-5,6-dihydroxy-1-naphthyl N-methylcarbamate) (accounted for 3.75-6.38% of the dose); UMET/11 (glucuronide of dihydro-dihydroxy-1-naphthyl N-methylcarbamate) (18.55%-28.46% of the dose); UMET/18 (α-naphthyl β-D-glucuronide sodium salt or α-naphthyl sulfate potassium salt (15.69-21.75 % of the dose); and UMET/23 (naphthyl sulfate) (17.78%-30.01% of the dose).

A total of 20 components was detected in the 24-hour feces by HPLC analysis. One component (FMET/15) was identified as parent and accounted for 0.2-1.4% of the administered dose by LC/MS technique. The remaining 19 components were not identified because the levels of radioactivity in these components were too low.

There were 2 major metabolites in the tissues from groups B-E at 6 hours after administration of ^{14}C-carbaryl. These metabolites were confirmed by LC/MS analysis as naphthyl sulfate (found in plasma, kidney, and urinary bladder) and naphthyl glucuronide (found in the kidney and urinary bladder). Quantitative identification for these metabolites was not available because the levels of radioactivity in these tissues were too low.

The sulfate conjugation pathway appears to be saturable following a subchronic (83-day) feeding of carbaryl at a high dose (group D, 7500 ppm). This saturation of the sulfate conjugation pathway is seen in the urinary levels of UMET/23 (naphthyl sulfate) between the dose groups following the 83-day dietary administration of non-radioactive carbaryl. The level of radioactivity associated with UMET/23 (naphthyl sulfate) was higher (23-27% of the dose) in 0, 250, and 1500 ppm dose groups and lower (12% of the dose) in the 7500 ppm group. On the other hand, the level of radioactivity associated with UMET/11 (naphthyl glucuronide) was lower (15-21% of the administered dose) in 0, 250, and 1500 ppm dose groups and higher (28%) in the group 7500 ppm group.

Statistically significant decreases ($p<0.05$ or $p<0.01$) in body weight (9-20%) when compared to the control group were observed only in the 7500 ppm group as early as study day 14 and sustained throughout the remainder of the study. In the 7500 ppm group, the statistically significant decreases ($p<0.05$ or $p<0.01$) in food consumption were observed at week 1 (74%), week 2 (61%), week 3 (40%), and weeks 4-11 (19-31%). In the 1500 ppm group, the statistically significant decreases ($p<0.05$) in food consumption were observed at week 5 (8%), week 10 (21%), and week 11 (12%).

Significant increases (statistical analyses were not performed) in kidney, spleen, and thyroid weights were observed in the 1500 or 7500 ppm groups when compared to the control group. Absolute and relative liver weights increased 18% and 39%, respectively, at 7500 ppm. Absolute spleen weight increased 30% at 7500 ppm and relative spleen weight increased 24% and 30% at 7500 and 1500 ppm, respectively. Absolute thyroid weight increased 63% and 69% at 7500 and 1500 ppm, respectively, and relative thyroid weight increased 103% and 121% at 7500 and 1500 ppm, respectively. Statistically significant increases ($p<0.01$) in total glutathione concentrations (higher by 79% per g of liver or 102% per g of protein) were observed at 7500 ppm only, compared to the controls.

The incidences of hepatocellular adenoma (benign) were 1/5, 0/5, 0/5, and 2/5 at 0, 250, 1500, and 7500 ppm, respectively. Although the authors concluded that "there was no treatment-related change in the incidence of tumors under carbaryl treatment," definite conclusion cannot be made from this finding based on the limited number of animals used.

Significant treatment-related changes were noted in liver, thyroid glands, and kidneys at 7500 ppm only. The incidences of centrilobular hypertrophy of the hepatocytes, pericholangitis (an inflammatory cell infiltrate around biliary ducts), and bile duct hyperplasia were 5/5, 3/5, and 3/5, respectively. The incidences of follicular cell hypertrophy of the thyroid glands were 0/5, 3/5, 5/5, and 5/5 and the incidences of transitional cell hyperplasia of the renal pelvis were 0/5, 0/5, 1/5 and 2/5 at 0, 250, 1500, and 7500 ppm, respectively.

This metabolism study in the rat is classified acceptable for its intended purpose of investigating "the mechanisms that caused the appearance of an increased incidence of tumors during the final year of a chronic dietary feeding study in the rat at the high dose level of 7500 ppm." Although the study supplies some information to the Agency, this study **does not satisfy** the guideline requirement for a metabolism study (85-1) in rats.

Metabolism - Special Study

The present investigation (MRID # 43832601) was conducted to identify and phenotype the potential for Carbaryl to induce hepatic cytochrome P-450 in male CD-1 mice following dietary administration of 8000 ppm Carbaryl in the diet. The data in this study represent results from mice used in a previous study (MRID # 432822-01) whose livers had been stored for biochemical analyses. These mice had received pre-treatment with 8000 ppm (1143 mg/kg/day) Carbaryl for 14 days. The results of biochemical analyses in the liver can be summarized as follows: Carbaryl pre- treatment produced significant increases in microsomal protein (132% of control), cytochrome P-450 (134% of control), ethoxyresorufin O-deethylase activity (190% of control), pentoxyresorufin O-dependentylase activity (313% of control), and increases in specific testosterone hydroxylase activities (6-alpha, 2ß-, 11ß,- and 16ß- hydroxylase activities). Taken together, these data appear to indicate a 'phenobarbital-type' of induction of liver xenobiotic-metabolizing enzymes as a result of Carbaryl pre-treatment at a high oral dose (1154 mg/kg/day). The similarity of the pattern of induction of liver xenobiotic-metabolizing enzymes by Carbaryl and phenobarbital is supported in part by literature data (Kelley et al., Biochem. Pharmacol. 15;(39)12:1991-1998). While this study provides useful information on the general type of induction observed after pretreatment with a high oral dose of Carbaryl, the actual relationship of induction to

Carbaryl toxicity was not addressed, as no metabolites of Carbaryl after this type of exposure were investigated. This study is classified as **acceptable** (non-guideline) and demonstrates the inductive effect of repeated high dose exposure to Carbaryl by the oral route.

Metabolism - Special Study

In a special study (MRID # 43282201), [1-^{14}C]-naphthyl-N-methylcarbamate (14-C carbaryl) was tested for the ability to bind to liver DNA in male CD1 mice treated with a single radiolabelled dose of carbaryl (75 mg/kg) or in mice pretreated with 8000 ppm (approximately 1143 mg/kg/day) unlabelled carbaryl in the diet for two weeks followed by a single 75 mg/kg radiolabelled dose. Binding of radiolabel to chromatin protein isolated from the livers of mice treated with a single dose or in pretreated mice was similar (specific activities ranging from 340.3-537.0 dpm/mg). No radioactivity was detectable in DNA samples isolated from mice treated with radiolabelled carbaryl (Covalent Binding Index < 0.1). According to the report, this maximum binding ability of carbaryl is more than 5 orders of magnitude below the Covalent Binding Index of aflatoxin B_1, and more than 4000 times lower than the Covalent Binding Index for 2-acetylaminofluorene. This study demonstrated the interaction of carbaryl with chromatin protein, but no significant interaction with DNA in the liver of male CD1 mice treated with either a single 75 mg/kg dose or in mice pretreated with 8000 ppm (1143 mg/kg/day) carbaryl in the diet followed by a single 75 mg/kg radiolabelled dose. This study was not conducted to satisfy a specific guideline requirement, but fulfills the purpose for which it was conducted.

870.7600 Dermal Absorption - Rat

Two dermal absorption studies in rats were conducted. In the study with a formulation containing 43.9% carbaryl (MRID 43552901), animals were exposed for 0.5, 1, 2, 4, 10 or 24 hours at doses of 35.6, 403 or 3450 µg/cm^2. Percent absorbed ranged from 2.14 to 24.9, 1.01 to 24.7 and 0.07 to 3.17 for the 35.6, 403 or 3450 µg/cm^2 doses, respectively (see Section 5.2 below). The HIARC determined that a 12.7% absorption (relative to an oral dose) should be used for risk assessment. This rate was based on the highest absorption rate at 10 hours, which is considered the duration of possible occupational exposure during a work day.

5.0 TOXICITY ENDPOINT SELECTION

5.1 See Section 9.2 for Endpoint Selection Table (page 51).

5.2 Dermal Absorption

Dermal Absorption Factor: 12.7 % from MRID 43552901 (findings discussed above)

The dermal absorption factor is required for long-term dermal and inhalation risk assessments since oral doses were selected for these exposure periods.

In a dermal absorption study (MRID # 43552901), radiolabeled ^{14}C-Carbaryl LXR Plus (43.9% a.i.) was applied to the skin of three groups of four male rats/group at doses of 35.6, 403 or 3450 µg/cm^2 for 0.5, 1, 2, 4, 10 or 24 hours. The ranges for percent of carbaryl absorbed for the 35.6, 403 and 3450 µg/cm^2 groups were 2.14-24.9, 1.01-24.7 and 0.07-3.17, respectively; the percent absorbed at 10 hours was 12.7, 7.44 and 1.93, respectively.

This study is classified as **Acceptable (guideline)** and **satisfies** the guidelines for a dermal absorption study.

In a dermal absorption study (MRID # 43339701), radiolabeled ^{14}C-Carbaryl Sevin (80.1% a.i.) was applied to the skin of three groups of four male rats/group at doses of 63, 626 or 3410 µg/cm^2 for 0.5, 1, 2, 4, 10 or 24 hours. The ranges for percent of carbaryl absorbed for the 63, 626 and 3410 µg/cm^2 doses were 0.66-16.6, <0.01-1.27 and 0.07-1.2, respectively; the percent absorbed at 10 hours was 8.90, 0.62 and 0.48, respectively.

This study is classified as **Acceptable (guideline)** and **satisfies** the guidelines for a dermal absorption study.

5.3 Classification of Carcinogenic Potential

5.3.1 The CARC concluded that carbaryl was carcinogenic to male mice at doses which were adequate and not excessive. Tumors in male and female rats and female mice, as well as other tumors in male mice, occurred at excessively toxic high dose levels. However, preneoplastic lesions in the target organs in male rats occurred at the mid dose level which was below the dose adequate for testing the carcinogenic potential of carbaryl. The findings of the rat combined chronic toxicity/carcinogenicity study are discussed below.

1. The reanalyses of rat tumor data showed that male rats had significant increasing trends and significant differences in pair-wise comparisons of the 7500 ppm dose group with the controls for thyroid follicular cell adenomas and combined adenomas/carcinomas, as well as for urinary bladder transitional cell papillomas, carcinomas, and combined papillomas/carcinomas, all at $p<0.01$. The increase in the incidence of combined thyroid follicular cell adenomas/carcinomas at

7500 ppm was driven by the adenomas. At 7500 ppm, the incidences of thyroid follicular cell adenomas, as well as combined urinary transitional cell papillomas and carcinomas, exceeded their respective range for the historical controls. The female rats had a significant increasing trend ($p<0.01$) and a significant increase by pair wise comparison of the 7500 ppm dose group with the controls for hepatocellular adenomas ($p<0.05$). The re-read of tumor data by the Pathology Working Group (PWG) showed that the female rats had a significant increasing trend for urinary bladder transitional cell papillomas, carcinomas and combined papillomas/carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls for urinary bladder transitional cell papillomas ($p<0.05$), carcinomas ($p<0.05$), and combined carcinomas/papillomas ($p<0.01$). The incidences of hepatocellular adenomas, urinary bladder transitional cell papillomas and urinary transitional cell carcinomas exceeded the respective ranges for the historical controls. The CARC noted that at the week 53 necropsy, transitional epithelial hyperplasia, a preneoplastic stage, was observed in the urinary bladder of mid dose tested (MDT) males and highest dose tested (HDT) males and females. After the 4-week recovery period, this change was still present in HDT males and females. At the terminal necropsy, the transitional cell hyperplasia was observed in HDT males and females, along with an increased incidence of squamous cell metaplasia, high mitotic index and atypia.

The HDT was judged to be excessive based on a significant ($p<0.5$) decrease in body weight gains during week 13 for males and females by 40% and 52%, respectively, as compared to controls. Decreased food efficiency and alterations in hematology and clinical chemistry values were also reported in both sexes at the high dose level. By weeks 52-53, plasma, RBC and brain cholinesterase (ChE) activities were significantly ($p<0.05$) decreased in males by 40%, 22% and 28%, respectively, and in females by 56%, 36% and 37%, respectively, as compared to controls. By week 104, plasma, RBC and brain ChE activities were significantly decreased in males by 42%, 30% and 9%, respectively, and in females by 46%, 38% and 22%, respectively.

The MDT was judged to be below the adequate dose for testing the carcinogenic potential of carbaryl. At this dose, there was no effect on body weight/body weight gain and only minor ChE inhibition (less than 20% inhibition of plasma, RBC and brain ChE in males and females at week 53, except for 26% inhibition of RBC in females; at week 105, only female RBC and brain ChE were decreased (22% and 16%, respectively). The CARC noted that the MDT male rats had transitional cell hyperplasia of the bladder, a preneoplastic lesion, at the week 53 necropsy. If the dose had been adequate, bladder tumors seen at the HDT may have occurred at the MDT.

2. The reanalyses of mouse tumor data showed that male mice had significant increasing trends in kidney tubule cell adenomas ($p<0.05$), carcinomas ($p<0.05$) and combined adenomas/carcinomas ($p< 0.01$). In mice, hemangiomas in the liver and spleen can progress to hemangiosarcomas. Therefore, the incidence of hemangiomas and hemangiosarcomas at various sites was combined and analyzed. There were significant differences ($p< 0.05$) in the pair-wise comparison of the $\geq$100 ppm (all doses tested) with the controls for hemangiosarcomas and in combined hemangiomas/hemangiosarcomas at 1000 and 8000 ppm. In addition, a significant difference in the pair-wise comparison of the 8000 ppm dose group with controls was noted for combined kidney tubule cell adenomas/carcinomas ($p< 0.05$).

The female mice had significant increasing trend in hepatocellular adenomas ($p< 0.01$), combined hepatocellular adenomas/carcinomas ($p< 0.01$), hemangiosarcomas ($p< 0.01$), and combined hemangiomas/hemangiosarcomas ($p<0.05$). There were also significant differences in the pair-wise comparison of the 8000 ppm dose group with the controls for hepatocellular adenomas ($p<0.05$), combined hepatocellular adenomas/carcinomas/hepatoblastomas ($p<0.01$), and hemangiosarcomas ($p<0.05$). Appropriate historical control data for various types of tumors were not available for comparison. However, based on recently submitted historical control data on vascular tumors in the liver and spleen (sites for most hemangiomas/hemangiosarcomas), the incidence of hemangiosarcomas exceeded the range for the historical controls in both male and female mice.

The CARC considered the dosing at the HDT in male and female mice to be excessive because the decrease in body weight gain, clinical signs and ChE inhibition, and histopathological changes in various organs were indicative of excessive toxicity. **The CARC concluded that the malignant vascular tumors (hemangiosarcomas) in male mice occurred at doses which were adequate and not excessive. In females these tumors occurred only at the highest dose which was excessively toxic. Nevertheless, the findings in female mice were supportive of vascular tumors in male mice.**

Carbaryl produces epoxides and its genotoxicity is manifested as chromosomal aberrations in cultured mammalian cells while older *in vivo* studies indicate negative results for aberrations. More recent studies with cultured cells have demonstrated effects on microtubule assembly, karyokinesis and cytokinesis as well as stress genes associated with oxidative damage.

5.3.2 Classification of Carcinogenic Potential

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified carbaryl into the category **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence considerations:

1. Carbaryl induced a statistically significant increase in urinary bladder tumors in male and female rats, thyroid tumors in male rats and liver tumors in female rats. These tumors were induced at an excessively toxic dose (7500 ppm) and, therefore, were not relevant for human cancer risk assessment. However, there was evidence of preneoplastic lesions in the bladder in males at 1500 ppm, a dose which was below the adequate dose for testing the carcinogenic potential of carbaryl. In mice, a treatment-related increase in malignant vascular tumors (hemangiosarcomas) was noted in males at all doses, both excessive and adequate, whereas in females, this same tumor type was seen only at excessive doses.

2. Carbaryl is clastogenic in *in vitro* studies with effects on aberrations; aneuploidy-associated events are also observed and further, a single report from the published literature suggests that carbaryl may induce oxidative stress. These types of effects may contribute to carbaryl-induced tumors. Nevertheless, carbaryl is negative for micronucleus induction in one mouse strain, not clastogenic in Syrian hamsters, and negative in a p53 knockout transgenic mouse bioassay.

5.3.3 Quantification of Carcinogenic Potential

The Committee recommended a low-dose linear extrapolation approach using all dose levels for the quantification of human cancer risk based on the most potent vascular tumors in mice. This approach was supported by the lack of confirmation of a mode of action. The Q_1*, based on the CD-1 mouse dietary study with ¾ Interspecies Scaling Factor, is 8.75×10^{-4} $(mg/kg/day)^{-1}$ in human equivalents.

6.0 FQPA CONSIDERATIONS

6.1 Degree of Concern Analysis and Residual Uncertainties

The HIARC concluded that there is no residual concern in the two-generation reproduction study because the dose-response effects in pups are well-characterized and the NOAEL for the offspring effects is above that was used for establishing the chronic Reference Dose (RfD) for chronic dietary risk assessment.

The HIARC selected the LOAEL of 3.1 mg/kg/day established in the chronic toxicity study in dogs for establishing the chronic RfD. Since a LOAEL was used, an additional uncertainty factor of 3X was applied (i.e, lack of a NOAEL) to the LOAEL. Although a NOAEL was not established in this study, the HIARC determined that a 3X was adequate (as opposed to a higher value) because: 1) cholinesterase inhibition in females was not accompanied by clinical signs; 2) no inhibition was seen for any cholinesterase compartment in males at this dose; 3) the magnitude of inhibition of plasma cholinesterase inhibition (12-23% decrease) was comparable to the magnitude of inhibition (22%) seen in the 5-week study in dogs indicating no cumulative effects following long-term exposure; 4) the study was well-conducted and there are sufficient data from subchronic and chronic duration studies in the other species which support cholinesterase inhibition as the critical effect.

In addition, based on the cholinesterase inhibition data, the dog appears to be more sensitive than the rat in long-term studies. Furthermore, use of the LOAEL of 3 mg/kg/day from the 1-year dog study with an uncertainty factor of 300 results in a NOAEL of 1 mg/kg/day. This extrapolated NOAEL is identical to that of the offspring NOAEL of 1.0 mg/kg/day established in the the developmental neurotoxicity study.

Thus, the NOAEL of 1 mg/kg/day used for establishing the chronic RfD is below the NOAEL of 5 mg/kg/day for offspring toxicity and the chronic RfD would be protective of the effects of concern for infants and children following chronic dietary exposures.

With regard to the developmental neurotoxicity study, the HIARC concluded that there was a low level of concern based on the following residual uncertainties

- The first uncertainty was the lack of a demonstrated effect level since morphometric measurements of brains in the offsprings were not performed at the mid-dose (1 mg/kg/day). However, this concern was negated since even at the high dose of 10 mg/kg/day, the morphometric changes were minimal and therefore, it is unlikely that adverse effects would be seen at 1 mg/kg/day, which is 10% of the LOAEL.

- The second uncertainty was the lack of comparative data in adults and offspring for cholinesterase inhibition. This concern was negated since no FOB alterations were seen in pups. Other studies in the data base have shown that when FOB alterations were seen in adult animals, they are usually accompanied with cholinesterase inhibition. Also, the results of the National Institute for Environmental Health Sciences study (discussed below) showed no difference in cholinesterase inhibition in pups and adults. There was a dose-related decrease in cholinesterase activity in the brain and blood of dams at gestation day 19 and fetuses taken at this time also showed a very similar level in fetal brain cholinesterase.

The HIARC concluded, that the NOAEL of 1 mg/kg/day selected for establishing the acute RfD would address the low level of concern for the residual concerns and would be protective of the effects of concern for infants and children following a single oral exposure.

6.2 Hazard Based- Special FQPA Safety Factor Recommendation

The HIARC concluded that the hazard based special FQPA safety factor should be reduced to 1x based on the following reasons:

1. The toxicology database is complete
2. There was no quantitative or qualitative evidence of increased susceptibility in rat or rabbit fetuses following *in utero* exposures
3. There was evidence of qualitative susceptibility and a low level of concern due to some residual uncertainties in the developmental neurotoxicity study. However, as discussed in Section I. 3, the acute RfD would address these residual uncertainties and would be protective of the pre-pre/post natal toxicity following an acute dietary exposure.
4. There was evidence of increased susceptibility in the offsprings in the two generation reproduction study, but there was no residual uncertainties. The chronic RfD would be protective of the pre-pre/post natal toxicity following chronic dietary exposures.
5. The dose selected for residential exposures, would be protective of the pre-pre/post natal toxicity following non-dietary exposures.

7.0 REFERENCES in MRID order

MRID 00148500. Acute Oral Toxicity - Rat. Cosmopolitan Safety Evaluation, Inc. Study Number 1290A, July 17, 1985. Unpublished

MRID 00148501. Acute Dermal Toxicity - Rabbit. Cosmopolitan Safety Evaluation, Inc. Study Number 1290B, June 8, 1985. Unpublished

MRID 00148502. Acute Inhalation - Rats. Cosmopolitan Safety Evaluation, Inc. Study Number 1290C, August 3, 1985. Unpublished

MRID 00148503. Primary Eye Irritation - Rabbit. Cosmopolitan Safety Evaluation, Inc., Study Number 1290D, May 3, 1985. Unpublished.

MRID 00148504. Primary Dermal Irritation - Rabbit. Cosmopolitan Safety Evaluation, Inc., Study Number 1290E, May 11, 1985. Unpublished.

MRID 00148505. Sensitization Study - Guinea Pig. Cosmopolitan Safety Evaluation, Inc., Study Number 1290F, June 9, 1985. Unpublished.

MRID No. 40166701. Hamada, N. (1987) One-Year Oral Toxicity Study in Beagle Dogs. Hazleton Laboratories. HLA 400-715, March 18, 1987. Unpublished.

MRID 41370301. Cifone, M. (1989) Mutagenicity Test on Carbaryl Technical in the in vitro Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay. Hazleton Laboratories America, Inc., Kensington, MD. Study Number 10862-0-447, November 22, 1989. Unpublished.

MRIDs 41370302 and 41420201. Young, R. (1989) Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells. Hazleton Laboratories America, Inc., Kensington, MD. Study Number 10862-0-435, November 6, 1989. Unpublished.

MRID 41370303. Lawlor, T. (1989) Mutagenicity Test on Carbaryl (Technical) in the Ames Salmonella/Microsome Reverse Mutation Assay. Hazleton Laboratories America, Inc., Kensington, MD. Study Number 10862-0-401, September 6, 1989. Unpublished.

MRID 41370304. Murli, H. (1989) Mutatgenicity Test on Carbaryl Technical in an in vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells. Hazleton Laboratories America, Inc., Kensington, MD. Study Number 10862-0-437, August 31, 1989. Unpublished.

MRID No. 42022801. Hamada, N. (1991) Subchronic Toxicity Study in Dogs with Carbaryl Technical. Hazleton Laboratories. HLA 656-152, March 28, 1991. Unpublished.

MRID No. 42786901. Hamada, N. (1993) Oncogenicity Study with Carbaryl Technical in CD-1®

Mice. Hazleton Washington, Inc., Vienna, VA. HWA 656-138., May 20, 1993. Unpublished.

MRID No. 42918801. Hamada, N. (1993) Combined Chronic Toxicity and Oncogenicity Study with Carbaryl Technical in Sprague-Dawley Rats. Hazleton Washington, Inc., Vienna, VA. HWA 656-139, September 7, 1993. Unpublished.

MRID 43282201. Sagelsdorff, P. (1994) Investigation of the Potential for Protein- and DNA-Binding of Carbaryl. CIBA-GEIGY Limited, Toxicology Services/Cell Biology, Basel, Switzerland. Study Number CB93/52, April 28, 1994. Unpublished.

MRID 43332101. Struble, C. (1994) Metabolism of 14-C Carbaryl in Rats (Preliminary and Definitive Phases). Hazleton Wisconsin, Inc. Study Number EC-92-222, August 5, 1994. Unpublished.

MRID 43339701. Cheng, T (1994) Dermal Absorption of (carbon 14)-Carbaryl (80S) in Male Rats (Preliminary and Definitive Phases). Hazleton Wisconsin, Final Report: Lab Project Number: HWI 6224-207. Unpublished.

MRID 43552901. Cheng, T (1995) Dermal Absorption of (carbon 14)-Carbaryl (XLR Plus) in Male Rats (Preliminary and Definitive Phases). Hazleton Wisconsin, Final Report: Lab Project Number: HWI 6224-206. Unpublished

MRID 43832601. Thomas, H. (1994) Carbaryl: Liver Cytochrome P-450 Inducer Phenotype in the Male CD-1 Mouse. Ciba-Geigy Limited, Toxicology Services, Basel, Switzerland. Laboratory Project Number: CB 94/23, October 21, 1994. Unpublished.

MRIDs 43845201 and 43845202. Brooks, W. *et al* (1995) An Acute Benchmark Dose Toxicity Study of Orally Administered Carbaryl, Technical Grade, in Rats. Bio-Research Laboratories, Ltd., Quebec, Canada. Study Number 97387, Unpublished.

MRID 43845203. Brooks, W. *et al* (1995) An Acute Study of the Time Course of Cholinesterase Inhibition by Orally Administered Carbaryl, Technical Grade. Bio-Research Laboratories, Ltd., Quebec, Canada. Unpublished.

MRID 43845204. Brooks, W. *et al* (1995) An Acute Study of the Potential Effects of a Single Orally Administered Dose of Carbaryl, Technical Grade, on Behavior and Neuromorphology in Rats. Bio-Research Laboratories, Ltd., Quebec, Canada. Study 97389. Unpublished.

MRID 44069301. Marshall, R. (1996). Carbaryl: Induction of Micronuclei in the Bone Marrow of Treated Mice. Corning Hazelton (Europe), Harrogate, North Yorkshire, England. Study No. 198/89/1052, March 13, 1996. Unpublished.

MRID 44122601. Robinson, K and B. Broxup (1996) A 13 Week Study of the Potential Effects of Orally Administered Carbaryl, Technical Grade, on Behavior, Neurochemistry and Neuromorphology in Rats. Bio-Research Laboratories Ltd., Senneville, Quebec. Laboratory Project I.D. 97390, September 24, 1996.

Unpublished

MRID 44393701. Robinson, K. and B. Broxup (1997) A Developmental Neurotoxicity Study of Orally Administered Carbaryl, Technical Grade, in the Rat. ClinTrials BioResearch, Ltd. Quebec, Canada. Laboratory Project I.D. 97391, September 23, 1997. Unpublished

MRID No. 44402501. Totis, M. (1997) Investigation of the Metabolism of ^{14}C-Carbaryl in the 15-Month-Old Male Rat Following Chronic Dietary Administration. Final Report. Rhone-Poulenc Agrochimie, Centre de Recherche, 355 rue Dostoievski, BP. 153, F-06903 Sophia Antipolis, France. Study No. 95288. Oct. 3, 1997. Unpublished

MRID 44732901. Repetto-Larsay, M. (1998) Carbaryl Developmental Toxicity Study in the Rat by Gavage. Rhône-Poulenc Agro Centre de Recherche, Sophia Antipolis, France. Study SA 98070, October 21, 1998. Unpublished

MRID 44904202. Tyl, RW; Marr, MC; Myers, CB. (1999) Developmental Toxicity Evaluation (with Cholinesterse Assessment) of Carbaryl Administered by Gavage to New Zealand White Rabbits. Reproductive & Developmental Toxicology Laboratory, Center for Life Sciences and Toxicology, Research Triangle Park, NC. RTI Identification No. 65C-7297-200/100, June 3, 1999. Unpublished.

MRID 44904204. Robinson, K. and Broxup, B. (1999) A Developmental Neurotoxicity Study of Orally Administered Carbaryl, Technical Grade, in the Rat. ClinTrials BioResearch Ltd., Senneville, Quebec, Canada. Laboratory Project I.D. 97391, June 1, 1999. Unpublished.

MRID 45281801. Chu zel F (1999). Carbaryl 6-Month Carcinogenicity Study in p53 Knockout Mice by Dietary Administration. Rhône-Poulenc Agro Centre de Recherche, Sophia Antipolis Cedex, France. Study Number SA98155, July 8, 1999. Unpublished.

MRID 45281802. Bigot D (1999). Validation on Transgenic Mice - p53 Knockout Mice - to Predict Rodent Carcinogenicity. Rhône-Poulenc Agro Centre de Recherche, Sophia Antipolis Cedex, France. Study Number SA97040, November 10, 1999. Published with the following citation: Carmichael NG, Debruyne EL, Bigot-Lasserre D (2000). The p53 heterozygous knockout mouse as a model for chemical carcinogenesis in vascular tissue. Environ Health Perspect 108(1):61-5.

MRID 45236603. Dan ge M (1998). Carbaryl, Prelimina ry 28-Day Toxicity Study in the Male TSG p53 Wild Type Mouse by Dietary Administration. Rhône-Poulenc Agro Centre de Recherche, Sophia Antipolis Cedex, France. Study Numbers SA 97499 and SA 97538, April 10, 1998. Unpublished.

MRID 45448101. Tyl, R., C. Myers, M. Marr. (2001). Two-generation reproductive toxicity evaluation of carbaryl (RPA007744) administered in the feed to CD® (Sprague-Dawley) rats. Reproductive and Developmental Laboratory, Center for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Life Sciences and Toxicology, Research Triangle Park, NC 27709. Laboratory report number 65C-07407-400, May 24, 2001. Unpublished.

MRID 45456701. Robinson K, and Broxup B. (2001) Final Report Amendment No. 2 Supplement to MRID 44393701- A Developmental Neurotoxicity Study of Orally Administered Carbaryl, Technical Grade, in the Rat. ClinTrials BioResearch Ltd., Quebec, Canada. Laboratory Project I.D. 97391. July 10, 2001. Unpublished.

MRID 45456702. Hamelin N, Yipchuck G. (2001) Morphometric Evaluation of Rat Brain Areas for Developmental Neuropathology. ClinTrials Bioresearch Ltd., Quebec, Canada. Laboratory Project I.D. 99579. July 9, 2001. Unpublished.

MRID 45456703. Robinson K, and Broxup B. (2001) Final Report Amendment No. 1 Supplement to MRID 44393701- A Developmental Neurotoxicity Study of Orally Administered Carbaryl, Technical Grade, in the Rat. ClinTrials BioResearch Ltd., Quebec, Canada. Laboratory Project I.D. 97391. July 6, 2001. Unpublished.

MRID 45630601. Austin, E.W. (2002). 4 Week Repeated-Dose Dermal Toxicity Study with Carbaryl Technical in Rats. Covance Laboratories, Madison, WI. Laboratory Study Identification Number 6224-268, March 8, 2002. Unpublished.

MRID 45630602. Austin, E.W. (2002). 4 Week Repeated-Dose Dermal Toxicity Study with SEVIN® XLR Plus in Rats. Covance Laboratories, Madison, WI. Laboratory Study Identification Number 6224-267, March 7, 2002. Unpublished.

MRID 45630603. Austin, E.W. (2002). 4 Week Repeated-Dose Dermal Toxicity Study with SEVIN® 80S in Rats. Covance Laboratories, Madison, WI. Laboratory Study Identification Number 6224-266, March 8, 2002. Unpublished.

9.0 APPENDICES
Tables for Use in Risk Assessment

9.1 Toxicity Profile Summary Tables

9.1.1 Acute Toxicity Table - See Section 4.1

9.1.2 Subchronic, Chronic and Other Toxicity Tables

Table 1: Toxicology Profile of Carbaryl

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100 90-Day oral toxicity rodents	N/A	
870.3150 90-Day oral toxicity in nonrodents	N/A	
870.3200 21/28-Day dermal toxicity with technical carbaryl	45630601(2002) acceptable/nonguideline 0, 20, 50, 100 mg/kg/day	systemic NOAEL = 20 mg/kg/day systemic LOAEL = 50 mg/kg/day based on decreased RBC cholinesterase in males and females and brain cholinesterase in males dermal NOAEL = 100 mg/kg/day dermal LOAEL not established
870.3200 21/28-Day dermal toxicity with Sevin® XLR Plus (44.82% a.i.)	45630602 (2002) unacceptable/nonguideline 0, 20, 50, 100 mcL/kg/day (0, 9.6, 24, 48 mg/kg/day)	systemic NOAEL = 50 mcL/kg/day (24 mg/kg/day) systemic LOAEL = 100 mcL/kg/day (48 mg/kg/day) based on decreased body weight gain dermal NOAEL = 100 mcL/kg/day (48 mg/kg/day) dermal LOAEL not established
870.3200 21/28-Day dermal toxicity with Sevin® 80S (80.07% a.i.)	45630603 (2002) unacceptable/nonguideline 0, 20, 50, 100 mg/kg/day	systemic NOAEL = 20 mg/kg/day systemic LOAEL = 50 mg/kg/day based on decreased RBC cholinesterase in males and females dermal NOAEL = 100 mg/kg/day dermal LOAEL not established
870.3250 90-Day dermal toxicity	N/A	
870.3465 90-Day inhalation toxicity	N/A	
870.3700a Prenatal developmental in rats	44732901 (1998) acceptable/guideline 0, 1, 4, 30 mg/kg/day (oral gavage)	Maternal NOAEL = 4 mg/kg/day LOAEL = 30 mg/kg/day based on clinical signs, decreased body weight gain (BWG) and food consumption Developmental NOAEL = 4 mg/kg/day LOAEL = 30 mg/kg/day based on decreased fetal body weight and incomplete ossification of multiple bones

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3700b Prenatal developmental in rabbits	44904202 (1999) Acceptable/guideline 0, 5, 50, 150 mg/kg/day (oral gavage)	Maternal NOAEL = 5 mg/kg/day LOAEL = 50 mg/kg/day based on decreased BWG and plasma cholinesterase inhibition (ChEI) Developmental NOAEL = 50 mg/kg/day LOAEL = 150 mg/kg/day based on decreased fetal weight
870.3800 Reproduction and fertility effects	45448101 (2001) acceptable/guideline 0, 75, 300, 1500 ppm (4.67, 31.34, and 92.43 mg/kg/day for F_0 males; 0, 5.56, 36.32, and 110.78 mg/kg/day for F_0 females; 0, 5.79, 23.49, and 124.33 mg/kg/day for F_1 males; and 0, 6.41, 26.91, and 135.54 mg/kg/day for F_1 females averaged over the premating period)	Parental NOAEL = 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females) Parental LOAEL = 1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females) based on decreased body weight, weight gain, and feed consumption Reproductive toxicity NOAEL is $\geq$1500 ppm (92.43-124.33 mg/kg/day for males and 110.78-135.54 mg/kg/day for females) Reproductive toxicity LOAEL not be established Offspring NOAEL = 75 ppm (4.67-5.79 mg/kg/day for males and 5.56-6.41 mg/kg/day for females). Offspring LOAEL = 300 ppm (23.49-31.34 mg/kg/day for males and 26.91-36.32 mg/kg/day for females) based on increased numbers of F_2 pups with no milk in the stomach and decreased pup survival.
870.4100a Chronic toxicity in rodents	N/A	
870.4100b Chronic toxicity in dogs	40166701 (1987) 0, 125, 400, 1250 ppm (0, 3.1, 10, 31.3 mg/kg/day) 42022801 (1991) 0, 20, 45, 125 ppm (5 weeks) (M: 0, 0.59, 1.43, 3.83; F: 0, 0,64, 1.54, 4.11 mg/kg/day) Together, the studies are Acceptable/guideline	MRID 40166701: NOAEL = not established in females LOAEL = 125 ppm based based on plasma and brain ChEI MRID 42022801: NOAEL = 45 ppm in males LOAEL = 125 ppm in males based on plasma ChEI

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.4200 Carcinogenicity in mice	42786901 (1993) Acceptable/guideline 0, 100, 1000 or 8000 ppm (M:0, 14.73, 145.99, 1248.93 mg/kg/day; F: 0, 18.11, 180.86, 1440.62)	systemic LOAEL = 1000 ppm based on increased intracytoplasmic droplets in bladder in males and females, chronic progressive nephropathy in males; NOAEL = 100 ppm RBC ChEI LOAEL for males = 1000 ppm , for females = 8000 ppm; NOAEL = 100 ppm for males, 1000 ppm for females plasma ChEI for males and females LOAEL > 8000 ppm; NOAEL ≥ 8000 ppm brain ChEI for males and females LOAEL = 8000 ppm; NOAEL = 1000 ppm increase in vascular tumors in all treated males and in females at 8000 ppm increase in adenomas, multiple adenomas, carcinomas of kidney in males at 8000 ppm increase in hepatic neoplasms (adenomas, carcinomas, one hepatoblastoma) in females at 8000 ppm
870.4300 Chronic Toxicity/ Carcinogenicity in rats	42918801 (1993) Acceptable/guideline 0, 250, 1500 & 7500 ppm (M: 0, 10, 60.2, 349.5 mg/kg/day; F: 0, 12.6, 78.6, 484.6 mg/kg/day)	systemic LOAEL = 1500 ppm in females based on decreased BW and BWG; 7500 ppm in males based on increased clinical signs, decreased BW, BWG and food consumption, increase in cataracts, clinical pathology changes, organ weight changes, nonneoplastic changes; NOAEL = 250 ppm in females and 1500 ppm in males plasma ChEI LOAEL = 7500 ppm in males and females; NOAEL = 1500 ppm RBC ChEI LOAEL = 1500 ppm in males and females; NOEL = 250 ppm brain ChEI LOAEL = 7500 ppm in males and females; NOEL = 1500 ppm at 7500 ppm, increase in liver adenomas in females, increase in benign transitional cell papillomas and transitional cell carcinomas in males and females, transitional cell carcinoma in kidney of one male, increase in benign thyroid follicular cell adenomas in males, follicular cell carcinoma in one male
Bacterial reverse mutation test 870.5100	41370303 (1989) Acceptable/guideline 5-1000 ug/plate	No evidence of mutagenicity in strains TA1535, TA 1537, TA1538, TA98 and TA100 with and without metabolic activation
In vitro mammalian chromosome aberration test (Chinese hamster ovary cells) 870.5385	41370304 (1989) Acceptable/guideline without S9 activation: 5-100 ug/mL, harvest at 20 hrs.; with S9 activation: 25-300 ug/mL, harvest at 30 hrs	Increase in chromosome aberrations with S9 activation
In vitro mammalian chromosome aberration test 870.5385	41370302; 41420201 (1989) Unacceptable/guideline S9 activation: 1-300 ug/mL in 3 trials; without S9 activation: 1-300 ug/mL in 2 trials	Results provide no clear indication of a mutagenic response, however study had several deficiencies

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
Mammalian erythrocyte micronucleus test 870. 5395	44069301 (1996) Unacceptable/guideline single oral gavage dose of 50, 100, 200 mg/kg	Carbaryl did not induce a clastogenic or aneugenic effect, however there was no convincing evidence that MTD was achieved
Unscheduled DNA synthesis 870.5550	41370301; 41810601 (1989) Acceptable/guideline 0.5 - 25.0 ug/mL	Negative
870.6200a Acute neurotoxicity screening battery in rats	MRID: 43845201-43845204 (1995) Acceptable/guideline 0, 10, 50, 125 mg/kg (oral gavage) Separate study for ChE: 0, 10, 30, 50 mg/kg; ChE done 1, 8, 24, 48 hrs post-dosing	Systemic LOAEL = 10 mg/kg based on decreased RBC, plasma, blood, brain ChE; NOAEL < 10 mg/kg
870.6200b Subchronic neurotoxicity screening battery in rats	MRID: 44122601 (1996) Acceptable/guideline 0, 1, 10, 30 mg/kg/day (oral gavage)	LOAEL for neurotoxicity = 10 mg/kg/day based on increased FOB changes; NOAEL = 1 mg/kg/day LOAEL for ChEI = 10 mg/kg/day based on decreased plasma, blood, RBC, brain ChE; NOAEL = 1 mg/kg/day
870.6300 Developmental neurotoxicity in rats	44393701 (1997) Acceptable/guideline 0, 0.1, 1.0, 10 mg/kg (oral gavage)	**Maternal** NOAEL = 1.0 mg/kg/day LOAEL = 10 mg/kg/day based on decreased BWG; FOB changes; RBC, plasma, whole blood, brain ChEI **Offspring** tentative NOAEL = 1.0 mg/kg/day tentative LOAEL = 10 mg/kg/day based on alterations in morphometric measurements (measurements were not done at lower doses)
870.7485 Metabolism and pharmacokinetics in rats	43332101 (1994) Acceptable/guideline 1 mg/kg (single and repeated oral doses; intravenous dose) and 50 mg/kg (single oral dose)	Absorption was complete at all doses. At 168 hrs., post-dose, negligible percentages of dose in any tissues. Kidney and blood contained highest concentrations of radioactivity. Excretion mostly through urine. A metabolic scheme with conjugated and non-conjugated metabolites was proposed.
870.7485 Metabolism and pharmacokinetics in rats	44402501 (1997) Acceptable/nonguideline 50 mg/kg (single oral radiolabeled dose); daily oral radiolabeled dose of 2 mg/kg for 7 days followed by 83 daily unlabeled doses of 0, 250, 1500 or 7500 ppm; males only	In all dosing regimens, urinary and fecal excretion was 93-103% of administered dose and tissue levels of radioactivity were minimal at 168 hrs. post-dosing. Two major metabolites in tissues at 6 hrs. post-dosing were naphthyl sulfate and naphthyl glucuronide, however quantitation was not possible. A total of 23 and 20 components were identified in the urine and feces, respectively. The sulfate conjugation pathway appears to be saturable following a 83-day feeding at 7500 ppm. BW and food consumption were decreased at 7500 ppm. Increases in kidney, spleen and thyroid weights were observed at 1500 and 7500 ppm. Non-neoplastic changes in liver, thyroids and kidneys were observed at 7500 ppm.

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.7600 Dermal penetration in rats	43552901 (1995) 43.9% a.i. Acceptable 35.6, 403, 3450 ug/cm^2	% absorbed at 10 hrs.: 12.7, 7.44 and 1.93 at 35.6, 403 and 3450 ug/cm^2, respectively
870.7600 Dermal penetration in rats	43339701 (1994) 80.1% a.i. Acceptable 63, 626, 3410 ug/cm^2	% absorbed at 10 hrs: 8.90, 0.62 and 0.48 at 63, 626 and 3410 ug/cm^2, respectively
Special studies in mice	43282201 (1994) Acceptable/nonguideline male mice: single radiolabeled dose of 75 mg/kg; pretreatment with 8000 ppm unlabeled carbaryl for 2 wks., then single radiolabeled dose of 75 mg/kg	Negative for DNA binding in liver
Special studies in mice	43832601 (1994) Acceptable/nonguideline continuation of MRID 43282201	in liver from mice treated at 8000 ppm, increase in microsomal protein, cytochrome P450, ethoxyresorufin O-deethylase, pentoxyresorufin O-dependentylase, and testosterone hydrolases indicates phenobarbital type of induction of metabolizing enzymes
Special study in mice	45281801, 45281802, 45236603 (1998-1999) Acceptable/nonguideline 0, 10, 30, 100, 300, 1000 and 4000 ppm (0, 1.8, 5.2, 17.5, 51.2, 164.5 and 716.6 mg/kg/day)	There was no evidence of neoplastic or preneoplastic changes in vascular tissue in heterozygous p53-deficient male mice treated with carbaryl for six months.

N/A Not Available

9.2 Summary of Toxicological Dose and Endpoints for CARBARYL for Use in Human Risk Assessment[1]

Table 2: Summary of Toxicology Endpoint Selection for Carbaryl

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Dietary Risk Assessments			
Acute Dietary general population including infants and children	NOAEL = **1** UF = **100** **Acute RfD** = **0.01** mg/kg/day	1	**Developmental Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on an increased incidence of FOB changes on the first day of dosing in maternal animals
Chronic Dietary all populations	LOAEL= **3.1** UF = **300** **Chronic RfD** = **0.01** mg/kg/day	1	**Chronic toxicity - dog** LOAEL = 3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.
Incidental Oral Short-Term (1 - 30 Days) Residential Only	NOAEL= **1** **MOE=** TBD	1	**Developmental Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on an increased incidence of FOB changes and decreases in RBC, whole blood, plasma and brain cholinesterase
Incidental Oral Intermediate-Term (1 - 6 Months) Residential Only	NOAEL= **1** **MOE** = TBD	1	**Subchronic Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on increased incidence of FOB changes; decrease in RBC, whole blood, plasma and brain cholinesterase.

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Non-Dietary Risk Assessments			
Dermal Short-Term (1 - 30 days)	Dermal NOAEL= **20**		**4-week dermal toxicity - rat** systemic LOAEL = 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females and brain cholinesterase in males.
Residential	**MOE** = TBD	1	
Occupational	100	1	
Dermal Intermediate-Term (1 - 6 Months)	Dermal NOAEL= **20**		**4-week dermal toxicity - rat** systemic LOAEL = 50 mg/kg/day based on statistically significant decreases in RBC cholinesterase in males and females and brain cholinesterase in males.
Residential	**MOE** = TBD	1	
Occupational	100	1	
Dermal Long-Term[a] (> 6 Months)	Oral NOAEL= **3.1**		**Chronic toxicity - dog** LOAEL = 3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.
Residential	**MOE** = TBD	1	
Occupational	300	1	
Inhalation Short-Term [b] (1 - 30 days)	Oral NOAEL= **1**		**Developmental Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on an increased incidence of FOB changes and statistically significant decreases in RBC, whole blood, plasma and brain cholinesterase
Residential	**MOE** = TBD	1	
Occupational	100	1	
Inhalation Intermediate-Term[b] (1 - 6 Months)	Oral NOAEL= **1**		**Subchronic Neurotoxicity - rat** LOAEL = 10 mg/kg/day based on increased incidence of FOB changes; decrease in RBC, whole blood, plasma and brain cholinesterase.
Residential	**MOE** = TBD	1	
Occupational	100	1	
Inhalation Long-Term[b] (>6 Months)	Oral NOAEL= **3.1**		**Chronic toxicity - dog** LOAEL = 3.1 mg/kg/day based on plasma and brain cholinesterase inhibition in females.

Exposure Scenario	Dose (mg/kg/day) UF /MOE	Hazard Based Special FQPA Safety Factor	Endpoint for Risk Assessment
Residential	**MOE** = TBD	1	
Occupational	300	1	
Cancer	**Classification:** **Q1* =** 8.75×10^{-4}		

a Since an oral NOAEL/LOAEL was selected, a dermal absorption factor of 12.7% should be used in route-to-route extrapolation.
b Since an oral NOAEL was selected, an inhalation factor of 100% should be used in route-to-route extrapolation.
TBD = To Be Determined. Target MOEs for residential exposures will be determined by the FQPA Safety Factor Committee.